The natural history, treatment strategies and clinical outcomes of HIV/HCV coinfection

Thesis presented for the degree of
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Declaration

The data analysed in this thesis are from the pan-European EuroSIDA study. EuroSIDA is run by a multidisciplinary team of data managers, IT support, statisticians and clinicians from across Europe. As a statistician based at UCL in London, my role in the team has not involved the collection or management of the large amount of data collected. However, I have played a role in cleaning and compiling the data for the use of statisticians in London.

For each analysis presented in this thesis, I formulated an idea and submitted a project proposal to the EuroSIDA steering committee. After receiving feedback and approval to go ahead with each project, I performed all statistical analyses and prepared poster and oral presentations for international conferences. Following final feedback at these conferences and from the EuroSIDA working group, I prepared manuscripts for publication in peer-reviewed journals and the chapters that appear in this thesis.

I, Daniel Grint confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

While the rate of AIDS-related death has declined, as a consequence of the effectiveness of antiviral treatment for HIV, HIV/HCV coinfection and in particular liver-related death (LRD) has assumed increasing importance. This thesis aims to analyse important epidemiological areas of HIV/HCV coinfection to improve the knowledge base of the subject and provide guidance to clinicians in a fast moving area of research.

Data for this thesis are from the EuroSIDA study, which is a large multi-centre pan-European prospective observational cohort study with over 18,000 HIV-positive individuals including approaching 5,000 HIV/HCV coinfected individuals. The study was initiated in 1994 and continues to expand and diversify to meet current research needs.

Results from the studies included in this thesis have shown that treatment for HIV in coinfected individuals can also have a beneficial effect on the natural course of HCV, with HCV viral load remaining stable over time in those treated for HIV compared with increasing HCV viral load in those not yet treated. The incidence of treatment for HCV has steadily increased in Europe to 4.7 per 100 PYFU in 2010, but remains low with just 25% of eligible patients receiving treatment. LRD accounts for more than a fifth of deaths in this population, with significant liver fibrosis and those triple infected with HBV at increased risk. The 5-year probability of LRD is low for those with F0/F1 fibrosis (2.2%), but increases substantially for those with F2/F3 (10.3%) and F4 (14.0%) fibrosis.

With potent new treatments for HCV coming to market, it is clear that while they remain prohibitively expensive they should be targeted at those at the greatest risk of LRD. The prognostic LRD score derived here will help clinicians to make difficult decisions on who should be prioritised for HCV treatment.

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Chapter 1

Thesis aims and analysis outline

1.1 Aims

The aims of this thesis were to describe epidemiological characteristics of HIV and hepatitis C (HCV) coinfected individuals from across Europe and to provide guidance on their optimal clinical management. Using data from the EuroSIDA study, five epidemiological studies are presented describing clinically important facets of HIV/HCV coinfection. Each study is outlined briefly below, in order of appearance, while the clinical importance and limitations of the findings are discussed in detail in each chapter. Chapter 9 draws on the whole body of work to discuss the overall significance and implications for the management of HIV/HCV coinfected individuals in Europe.

1.2 Outline of thesis

1.2.1 Chapter 2

Introduction to HIV and HCV coinfection

This chapter provides a thorough introduction to both HIV and HCV as independent viruses and HIV/HCV in coinfection. Details of the discovery and nature of the viruses, the changing epidemiology of infection over time, modes of infection, the clinical manifestation of infection and the history of treatment are discussed in depth.

1.2.2 Chapter 3

Data and statistical methodology

This chapter introduces the EuroSIDA study, which is where the data analysed in this thesis are taken from, describing the origin of the study along with the spectrum of data collected and monitoring efforts to ensure data integrity. Also discussed are the statistical methods used throughout the analysis in this thesis, detailing statistical models used along with exploratory analysis and model building strategies.

1.2.3 Chapter 4

The natural history of HCV RNA during chronic HCV infection among HIV/HCV coinfected individuals

This chapter focuses on HCV viral load and how it changes over time in chronically infected HIV/HCV coinfected individuals. The aim of this chapter was to describe the natural history of HCV RNA in coinfected individuals and to identify factors associated with baseline HCV RNA and changes over time. The main statistical method used in this analysis was a random effects mixed model to model each individual's HCV RNA profile accounting for within subject variability.

The data presented in this chapter were originally presented as an oral abstract at the European AIDS Clinical Society (EACS) conference in Belgrade, October 2011. The analysis was then finalised and published in-part in HIV Medicine in February 2013. The published paper can be seen in Appendix III.

1.2.4 Chapter 5

Temporal changes and regional differences in the uptake of treatment for HCV among HIV/HCV coinfected individuals in EuroSIDA

This chapter documents the rate of uptake of treatment for HCV among HIV/HCV coinfected individuals between the years 1998 and 2010. As mentioned in Chapter 2 Section 2.2.6, gold standard HCV therapy over the follow-up period for this chapter consisted of pegylated-interferon plus ribavirin[1]. The aims of this chapter were to describe the rate of HCV treatment uptake among coinfected individuals and to highlight regional differences in Europe. Further, this study aimed to identify whether individuals with significant liver fibrosis, in the greatest need of HCV therapy, were being selected for treatment. Poisson regression was used to model the incidence of HCV treatment uptake over time.

The data presented in this chapter were originally presented as an oral abstract at the 11th International Conference on Drug Therapy in HIV in Glasgow, November 2012. The analysis was then finalised and published in-part in HIV Medicine in May 2013. The published paper can be seen in Appendix IV.

1.2.5 Chapter 6

The incidence of antiretroviral drug discontinuation among HIV/HCV coinfected individuals and those with significant liver fibrosis

This chapter focuses on the relationship between antiretroviral (ARV) drug discontinuation and HIV/HCV coinfection. The aims of this study were to describe the incidence of ARV drug discontinuation in HIV monoinfected and HIV/HCV coinfected individuals to see if drug discontinuation was more common among coinfected individuals. A further aim of this study

was to describe the association between significant liver fibrosis and the rate of ARV drug discontinuation, while identifying the drug classes and individual drugs most likely to be discontinued. The main statistical method used in this study was a Poisson regression model using generalised estimating equations to model the rate of ARV drug discontinuation accounting for within subject variability.

The data presented in this chapter were originally presented as a poster abstract at the 20th Conference on Retroviruses and Opportunistic Infections (CROI) in Atlanta, March 2013. The analysis was then finalised and published in-part in AIDS in September 2013. The published paper can be seen in Appendix V.

1.2.6 Chapter 7

Liver-related death among HIV/HCV coinfected individuals, what are the implications for treatment with direct-acting antivirals?

This chapter focuses on causes of death among HIV/HCV coinfected individuals. The aims of this study were to document causes of death among coinfected individuals in Europe and to describe changes in the rate of liver-related death (LRD) over time. Further, as it has become clear that new direct-acting antiviral treatments for HCV will be prohibitively expensive[2], this study aimed to identify factors associated with progression to LRD so that those at the highest risk could be prioritised for treatment. In this study Poisson regression is used to model the incidence rate of LRD over time. Cox proportional hazards regression, using the Fine and Gray methodology for handling competing risks, was used to identify factors associated with LRD.

The data presented in this chapter were originally presented as a poster abstract at the 21st Conference on Retroviruses and Opportunistic Infections (CROI) in Boston, March 2014. The analysis was then finalised and published in-part in AIDS in February 2015. The published paper can be seen in Appendix VI.

1.2.7 Chapter 8

A validated prognostic score for estimating the risk of liver-related death among HIV/HCV coinfected individuals

This chapter builds on the work of the previous chapter by creating a prognostic score for progression to LRD among HIV/HCV coinfected individuals. The aims of this analysis were to identify factors associated with progression to LRD among HIV/HCV coinfected individuals and to create a simple, easily applicable prognostic score to identify those at the greatest risk of LRD. The prognostic score was developed using data from the EuroSIDA

study and then validated using data from the Swiss HIV Cohort Study. The main statistical method used in this analysis was a Cox proportional hazards regression model, using the Fine and Gray methodology for handling competing risks, to identify factors associated with LRD using stepwise variable selection. The prognostic score was derived from the coefficients of the variables retained in the model.

The data presented in this chapter were originally presented as a poster abstract at the 22nd Conference on Retroviruses and Opportunistic Infections (CROI) in Seattle, February 2015. The analysis has been subsequently finalised with the aim of submitting for publication at CID in the summer of 2015.

1.2.8 Chapter 9

Overall significance and conclusions

This chapter draws on the whole body of work in this thesis to underline the key conclusions and implications for the clinical management of HIV/HCV coinfection. Key limitations of the EuroSIDA data and analysis presented in this thesis and are discussed in detail along with opportunities for further work.

Chapter 2

Introduction

2.1 Human Immunodeficiency Virus (HIV)

The human immunodeficiency virus (HIV) epidemic has changed markedly over its 30-year history. It was discovered when young gay men started dying of obscure conditions in the United States and has grown from a few small studies of a few infections to a well-documented worldwide pandemic[3-5]. Scientific advancements mean that today HIV is seen as a chronic disease which can be controlled with appropriate treatment[6;7]. As such HIV-positive people in resource rich settings now have life expectancy approaching that of those without the condition[8;9]. However, when HIV first appeared in the early 1980s little was known about the virus and infection would lead to death within a short time of developing an AIDS-defining condition[10].

Although potent treatment for HIV infection now exists there are large discrepancies in the availability and uptake of treatment and HIV remains one of the leading causes of death worldwide[11;12]. Further, the number of cases continues to increase in many regions and wealth settings[13]. Consequently, although gains have been made over the past decade, mortality rates remain comparatively high in resource-limited settings life expectancy is estimated to be reduced by 10-20 years[14;15].

Whereas the development of successful on-going treatment for HIV has proved fruitful, the search for an effective vaccine and a cure has been less successful[16-18]. Although research continues in these areas the reality is that HIV-positive people face a lifetime of treatment which places a substantial burden on their lives[16]. Further, treatment is not without complications with many of the available drugs associated with toxicities and adverse events[19-21], although toxicity profiles are better with newer drugs[22]. More recently it has also emerged that HIV infection may be associated with an increased risk of death from causes not directly related to HIV[23-26], while there may also be an inflammatory effect of having the HIV virus circulating in the body[27]. Consequently, research continues with the aim of improving the lives of those living with HIV.

2.1.1 The Beginning of the Epidemic

Although the cause was unknown at the time, the first cases of HIV and AIDS have been identified from samples dating back to the 1950s[28]. A sample taken from a male from the Democratic Republic of the Congo in 1959 is the first identifiable evidence of HIV[28]. The AIDS epidemic was formally recognised by health professionals in 1981 when previously healthy young gay men in the United States started to die with alarming regularity from conditions that had rarely been seen before[3;29-31], such as the rare cancer Kaposi's sarcoma and pneumocystis carinii pneumonia (PCP)[6;29-36]. Initially there was a lot of homophobic stigma associated with these symptoms but soon injecting drug users (IDU), haemophiliacs and others who had received blood transfusions started to present with similar symptoms[37-40;40-45]. Around the same time similar cases started to appear in Europe, also in gay men and in those to have received blood products or injected drugs[46-51]. Further, it soon became apparent that children to parents from these risk groups were also presenting with these rare conditions[52;53].

Africans also began to report similar cases with a large increase in the number of Kaposi's sarcoma cases[54-56]. However, in Africa cases were not restricted to gay men and injecting drug users, giving rise to the idea of a secondary epidemic not restricted to any particular risk groups[56;57].

The cause of these rare diseases was soon discovered to be severe immunodeficiency[3;32;33]. Immunodeficiency was a rare condition but not unheard of at the time, however, in these new cases recovery never occurred and the mortality rate was 100%, which was both shocking and worrying[3]. In 1982, the Centers for Disease Control and Prevention (CDC) named the condition Acquired Immunodeficiency Syndrome (AIDS)[40].

2.1.2 Discovery of the HIV Virus

The HIV virus was discovered in 1983 along with the knowledge that it was responsible for the development of AIDS. A French team at the Pasteur institute and the American Robert Gallo and collaborators both discovered the virus at around the same time in separate locations. The Pasteur Institute published a paper in 1983 stating that they had isolated a new virus from a person at risk of AIDS which the authors called Lymphadenopathy Associated Virus (LAV)[58;59]. The next year Robert Gallo published a series of papers describing a new retrovirus which he named Human T-cell Lymphotropic Virus 3 (HTLV-III) and demonstrated that it was the cause of AIDS[60-62]. It was soon discovered that these two viruses were in fact the same and due to the effect of the virus on the human immune system it was renamed Human Immunodeficiency Virus (HIV)[3;63;64]. Further advances

continued throughout the 1980s with the discovery that HIV actually took two distinct forms, HIV-1 and HIV-2, which were not closely related[3]. Both viruses were also found to exhibit extensive genetic diversity with a number of distinct subtypes and clades identified[65].

2.1.2.1 HIV in Humans

How HIV came to infect humans was a topic of much speculation to begin with. Many wild and accusatory theories were discussed ranging from a contaminated polio vaccine in Africa to a biological weapon of mass destruction developed in the United States[66;67]. These theories were dismissed when a virus very similar in nature to HIV was discovered in chimpanzees prior to the turn of the millennium[68]. It is now known that HIV-1 passed from chimpanzees to humans many times[68-70], most likely during the preparation of food and in unintended blood-to-blood contact[71], which has resulted in the wide-ranging genetic diversity of the virus[65;72;73]. Similarly, HIV-2 is almost identical to Simian Immunodeficiency Virus (SIV) which is found in sooty mangabey monkeys[65;74].

HIV has existed as a human virus for a little more than 100 years and almost certainly originated in Africa, with retrospective studies identifying the virus as early as the late 1950s[75;76]. The diversity of these early strains of HIV, in comparison to the strains that initially crossed over into humans, leads us to believe that HIV had been present in the human population for many years before even these early studies have identified it[28]. It is thought that the first human HIV infection probably occurred in Central Africa around 1930, when the first townships were developing in the area[69;75;77]. AIDS can first be identified in Africa from medical records in the 1950s, however, as the existence of the HIV virus was unknown at that time it is highly likely that AIDS-related deaths occurred earlier but were undiagnosed[76;78;79].

HIV-1 is more infectious than HIV-2 and is responsible for the majority of prevalent cases and new infections worldwide[3;77;80]. In the developed world, HIV-1 predominates in high risk groups such as men who have sex with men, injecting drug users and sex workers[81]. In the United States it is estimated that the HIV-1 epidemic began sometime in the late 1960s[79;82]. AIDS-defining conditions were prevalent in approximately 4.5% of gay men in San Francisco and 6.6% of gay men in New York in 1978[83;84], which due to the long interval between initial HIV infection and the development of AIDS-defining conditions, suggests that HIV-1 had been present in the population for some time[79]. In Europe, the first record of AIDS occurred as early as 1959 when a sailor was reported to die from an AIDS-related condition, considered to be one of the earliest records of AIDS in the developed world[85].

As HIV-2 is less infectious than HIV-1 it has spread at a slower rate and as such it is easier to determine the origin[3]. HIV-2 accounts for a fraction of the worldwide burden of HIV infection and is predominantly found in West Africa, originating in Guinea Bissau[3;81]. These regions are also home to sooty mangabey monkeys, who are known to have been infected with SIV[65;74]. Transmission of HIV-2 from monkey to human is thought to have occurred in the home in West Africa where they are commonly kept as pets or food[65].

The existence of networks of gay men and injecting drug users meant that HIV was able to spread rapidly through North America and Europe during the 1980s[79]. Meanwhile, in Africa a massive increase in antibiotic injections was taking place[86], which coupled with exponential growth in worldwide travel led to the development of a worldwide epidemic and a dramatic increase in the number of AIDS-related deaths[3;87].

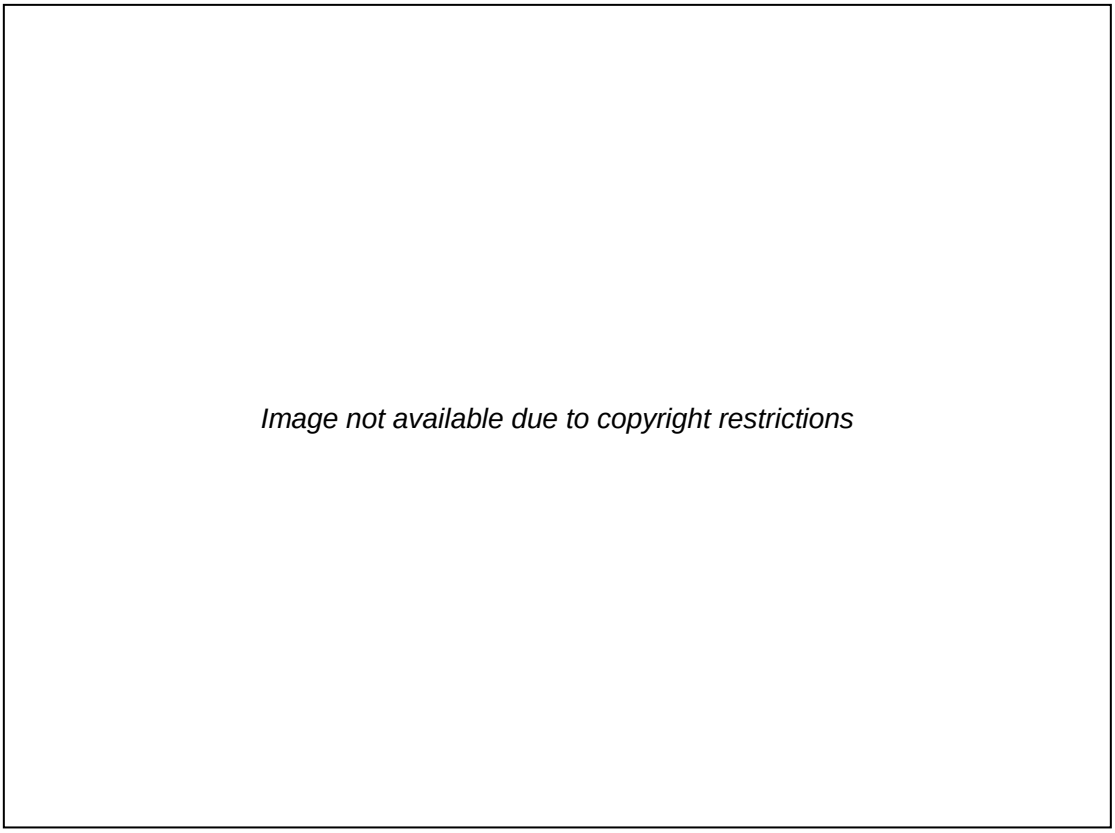
2.1.3 The HIV Virus

HIV is a retrovirus that belongs to a group of viruses called lentiviruses[65;88]. Retroviruses require help from the cells of a host to replicate and are known for long latent periods between infection and the development of symptoms and eventually death[12]. HIV targets CD4 lymphocyte cells to act as a host for viral replication[89]. These cells are a vital component of the human immune system and their depletion leads to the onset of AIDS[90;91]. The HIV lifecycle inside the body is shown in Figure 2.1.1[87].

After HIV has made its way into the blood stream of a host it begins to search for CD4 lymphocyte cells. When it encounters a CD4 cell it binds to the surface using the CCR5 or CXCR4 receptors, in a process known as fusion, and releases HIV RNA[92]. In the host CD4 cell, with the help of the HIV reverse transcription enzyme, HIV RNA is converted to DNA compatible with the host's human DNA[93]. Through a process known as integration, the HIV DNA is then combined with the host DNA[3]. Once the HIV DNA has integrated with the host it is treated just as any other human gene[87]. Using human enzymes and a process named transcription, long strands of HIV RNA containing full copies of HIV's genetic material are produced in the cell nucleus[94;95]. These new long HIV RNA strands are then carried outside the nucleus and the human protease enzyme translates them into small pieces of protein essential for building new HIV virons[94;95]. Once these small building blocks come together to form new HIV virons they bud off the host cell and re-enter the blood stream in search of new CD4 cells to repeat the process over again[94;95].

Once infected with HIV, a single CD4 cell can produce many thousand new HIV virons[12]. Further, the host cell does not survive the process as it is significantly weakened by the integration of HIV DNA[94]. This exponential build-up of HIV virons in the blood stream and

Figure 2.1.1 The HIV life cycle[87]

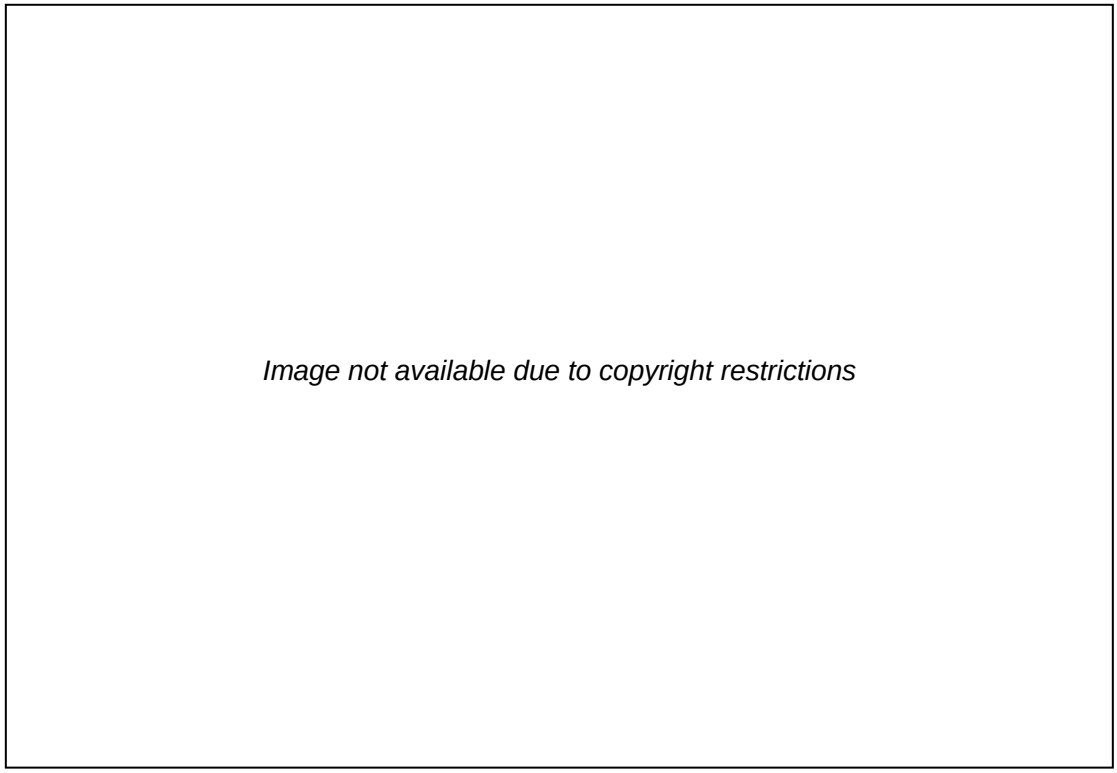


subsequent depletion of CD4 cells results in the eradication of the human immune system and leads to AIDS and death if left untreated[91]. It's also possible for CD4 cells infected with HIV to lie dormant for many years and begin to produce HIV virons when activated at a later time[96]. These latent HIV infected particles, known as HIV reservoirs, mean that attempting to cure HIV is a very complicated process and as a result treatment remains a lifelong commitment[97].

2.1.4 HIV and the Immune System

HIV infection leads to the death of CD4 cells while circulating HIV in the blood stream inhibits the body's ability to replace them[89]. CD4 cells counts vary considerably due to a number of natural factors including exercise and stress, but in healthy individuals they are usually within the range 500-1600cells/mm^3 [98-101]. Among HIV-positive individuals CD4 cell counts are often much lower and in the absence of treatment CD4 cell counts will continue to decrease as a consequence of HIV viral replication taking place in the body[99;102]. Figure 2.1.2 shows the decline in CD4 cell count associated with prolonged untreated HIV infection. After a sharp decline in CD4 cell count during the first few weeks of infection, the CD4 cell count stabilizes as the immune system manages to check the rapid replication of HIV RNA[103]. However, the CD4 cell count will continue to decrease slowly over time to very low levels by 10 years after initial infection[103]. Once CD4 cell counts drop below

Figure 2.1.2 The natural history of HIV infection, HIV viral load and CD4 cell count[103]



200cells/mm^3 there is a very high risk of developing an AIDS-defining condition, which will eventually lead to death[104-107].

Difficulties in studying the immune system in living people have proved a major stumbling block in understanding and explaining how the HIV virus directly impacts human CD4 immune cells[89]. However, technology for measuring CD4 cell counts has been readily available for some time and remains relatively cheap, costing approximately $5 in the developing world and $60 in the developed world[108]. Methods to measure the amount of HIV RNA circulating in the blood stream became available in 1996 and have since been used alongside CD4 cell counts to classify each individual's HIV infection status[109-111]. During the first few weeks of HIV infection viral loads can reach levels in the tens of millions per ml of blood, coinciding with a steep decline in CD4 cell count, before falling to more stable levels[103]. Similar to low CD4 cell count, many studies have documented an association between high HIV viral load and faster progression to AIDS and death[109;110;112;113].

HIV RNA tests are more expensive than CD4 cell counts, costing approximately $25 in the developing world and $120 in the developed world[108]. While both measures are important predictive markers of disease progression, CD4 cell count is thought to be a better short term marker of AIDS progression or death[114-118], while HIV viral loads are considered good long term markers of disease progression[110;114;119;120]. However, in the modern era where

the costs of treatment and monitoring are constantly being re-evaluated, many studies have attempted to assess the impact of a reduction in the frequency of viral load and CD4 monitoring, often concluding that less frequent testing is unlikely to lead to a significant increase in mortality or drug resistance[121;122].

2.1.5 The Clinical Stages of HIV Disease Progression

Classification systems for the stages of HIV disease and the progression to an AIDS-defining condition have been developed by the world health organisation (WHO) and the centres for disease and control (CDC). The CDC system relies on CD4 cell count monitoring and recognition of conditions associated with those with HIV, while the WHO system can be used in all resource settings, where CD4 cell count monitoring may not be available, and provides a description of conditions specific to the different stages of HIV infection[123;124]. Table 2.1.1 shows the stages of each classification system along with the clinical manifestations and relevant CD4 cell counts associated with each level.

HIV seroconversion

The first weeks of infection with HIV are referred to as primary or acute infection, seroconversion takes place in this period and is the process by which an individual's

Table 2.1.1 Classification of stages of HIV infection

WHO Stage	***CDC Stage***	***Clinical Monitoring[123]***	***CD4 Monitoring[124]***
1: Asymptomatic	A: Asymptomatic	No HIV-related symptoms	CD4 cell count ≥500cells/mm^3
2: Mild	B1: Symptomatic High CD4	Unexplained weight loss, Respiratory infections, Herpes zoster, Oral ulceration, Seborrhoeic dermatitis, Fungal nail infection	HIV-related symptoms And CD4 cell count ≥500cells/mm^3
3: Advanced	B2: Symptomatic Declining CD4	Severe weight loss, Chronic diarrhoea, Persistent fever, Oral candidiasis, Pulmonary TB, Severe bacterial infection	HIV-related symptoms And CD4 cell count 200-499cells/mm^3
4: Severe (AIDS)	C: AIDS	HIV wasting syndrome, Pneumonia, Chronic herpes simplex, Extrapulmonary TB, Kaposi sarcoma, HIV encephalopathy	AIDS-defining conditions And CD4 cell count <200 cells/mm^3

immune system recognises HIV and develops an immune response. Only once seroconversion is complete will a person test positive for HIV antibodies[125]. During primary infection HIV replicates rapidly infecting many CD4 cells and very high levels of HIV RNA are often present in the blood stream, potentially reaching millions of copies of the virus per millilitre of blood[12;126-128]. As shown in Figure 2.1.2 this results in a substantial, but transient, reduction in the number of CD4 cells and many people will experience flu-like symptoms[126-129]. After approximately 6 weeks, primary infection will end and HIV viral load will begin to decline, coinciding with a rebound in the level of CD4 cells. The majority of people will then move to a period of clinical latency, experiencing no sign or symptoms of HIV infection for many years[128;129].

Clinical latency

As shown in Figure 2.1.2, the period of clinical latency is categorised by a gradual reduction in the number of CD4 cell counts[12]. The immune system can cope with reducing CD4 cell counts for a median of 10 years following infection with HIV, with some able to survive for as long as 20 years without the need for treatment[130]. However, without treatment, older people are more likely to experience disease progression due to natural fluctuations in CD4 cell count according to age[12].

It is estimated that the gradual reduction in CD4 cell count during the phase of clinical latency sees the loss of approximately 50-90 CD4 cells per ml each year, while a CD4 cell count of 500 represents an immune system with approximately half as many CD4 cells as a healthy HIV-negative person[12;131;132]. However, in the period 18-24 months before the development of an AIDS-defining condition, the rate of CD4 cell depletion increases 3- to 5-fold[131;132].

It appears that the human immune system can function reasonably well with CD4 cell counts down to 200cells/mm^3, as opportunistic infections are rarely seen in those with CD4 ≥200cells/mm^3. However, once CD4 cell count levels drop as low as 50cells/mm^3 there is a very high risk of opportunistic infection, complications and death[100].

AIDS

The development of an AIDS-defining condition signals the last stage of HIV disease. Table 2.1.2 shows the full list of AIDS-defining conditions according to the CDC[133]. The CDC also recognises a CD4 cell count <200cells/mm^3 in an HIV-positive person to be an AIDS-defining condition[124]. Life expectancy following diagnosis with an AIDS-defining condition will vary depending on each specific condition, however, without adequate treatment it has been reported that the median survival time following an AIDS diagnosis is between 3 and

Table 2.1.2 CDC list of AIDS-defining conditions[133]

AIDS-defining conditions:
Bacterial infections, multiple of recurrent
Candidiasis of the bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive
Coccidioiodomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex: chronic ulcers (>1 month's duration), or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi sarcoma
Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis of any site, pulmonary, disseminated or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jiroveci pneumonia (PCP)
Pneumonia, recurrent
Progressive multifocal leukoencephalopathy
Salmonella septicaemia, recurrent
Toxoplasmosis of brain, onset at age >1 month
Wasting syndrome attributed to HIV

50 months[134]. Further, the length of survival is thought to halve at the diagnosis of a second AIDS-defining condition[134].

2.1.6 The global HIV epidemic

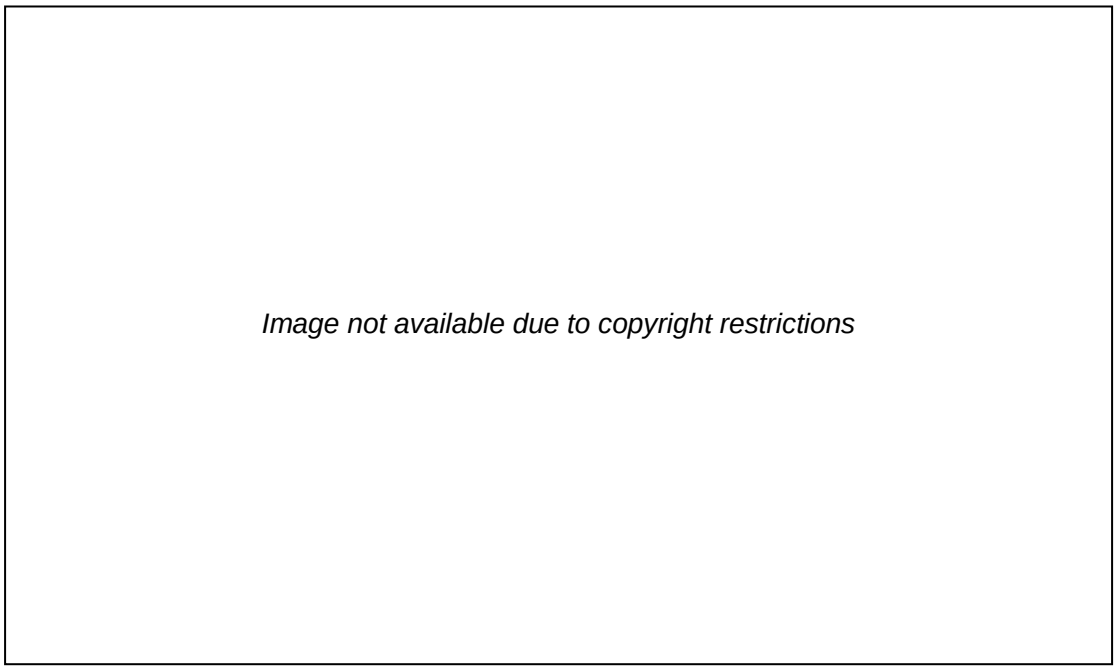
In 2012 UNAIDS reported on the worldwide distribution of the burden of HIV and the global AIDS epidemic. The report shows that there were an estimated 34.0 million (95% C.I. 31.4 million – 35.9 million) people living with HIV at the end of 2011[135]. The authors estimate that 0.8% of adults aged 15-49 years are living with HIV worldwide, however the burden of the epidemic varies considerably between countries and regions[135].

The most severely affected region continues to be Sub-Saharan Africa, where approaching 1 in 20 adults (4.9%) are living with HIV, which accounts for 69% of the total number of people living with HIV worldwide[135]. The second largest affected region is South-East and East Asia, where approaching 5 million people are living with HIV[135]. In terms of prevalence, following sub-Saharan Africa, the regions most heavily affected are the Caribbean, Eastern Europe and Central Asia, where 1.0% of adults were living with HIV in 2011[135]. Figure 2.1.3 from the WHO shows a graphical representation of the prevalence of HIV infection across the world in 2010 and mirrors the estimates from UNAIDS.

New infections declining

The global peak incidence of new HIV cases was thought to have occurred in 1996, with an estimated 3.5 million new infections[13]. Although the number of patients infected worldwide

Figure 2.1.3 Global view of the HIV epidemic in 2010[136]



with HIV is substantial, the number of people newly infected is falling per year. According to the UNAIDS report, the estimated number of people acquiring HIV infection in 2011 (2.5 million (2.2 million – 2.8 million)) was 20% lower than in 2001, although here too, variation is considerable between countries and regions[135]. Since 2001, the fastest declines in the number of people acquiring HIV infection have occurred in the Caribbean (42%) and sub-Saharan Africa (25%)[135]. However, in other parts of the world HIV trends remains a cause for concern. Since 2001, the number of people newly infected in the Middle East and North Africa has increased by more than 35% from 27,000 to 37,000 per year[135]. Data also indicates that the incidence of HIV infection in Eastern Europe and Central Asia began to rise in the late 2000's after remaining relatively stable for several years[135]. Figure 2.1.4 below from the WHO shows a graphical representation of the number of new HIV infections per year globally in 2010 and mirrors the estimates from UNAIDS.

Many national epidemics have changed dramatically over the past decade. In 23 countries in sub-Saharan Africa the incidence of HIV infection has reduced by more than 25%. However, despite these improvements sub-Saharan Africa continues to account for 71% of the adults and children newly infected in 2011, which highlights the importance of efforts to improve HIV prevention in the region[135]. Before the HIV epidemic took hold in sub-Saharan Africa, progress was being made in general health with the average life expectancy reaching 62 years, while it was hoped further gains would see the region soon approach the level of life expectancy seen in the developed world. However, the HIV epidemic has

Figure 2.1.4 Estimated number of new HIV infections worldwide in 2010[136]

Image not available due to copyright restrictions

had a devastating effect on the region and in 2005 the estimated life expectancy in sub-Saharan Africa had fallen to 44 years[137].

With the exception of sub-Saharan Africa, the HIV epidemic is predominantly confined to IDUs, MSM and sex workers[5]. In sub-Saharan Africa the prevalence is so high and widespread that everyone is considered at risk of infection[5;138]. Further, as transmission in sub-Saharan Africa is mostly driven by heterosexual contact and mother-to-child transmission, it is also the only region where there is a higher prevalence among women[5;138].

2.1.7 The European HIV Epidemic

HIV infection is of major public health importance in Europe. In 2011, HIV/AIDS surveillance in Europe reported that 53,974 HIV diagnoses were reported by 50 of the 53 countries in the WHO European Region[139]. The surveillance results suggest that HIV transmission continues in many countries, with an overall rate of 7.6 diagnoses per 100,000 population[139]. Figure 2.1.5 shows the number of new HIV infections by region of Europe and year of diagnosis.

There are considerable regional differences in the HIV epidemic within Europe, with the prevalence and incidence of infection varying from country to country. The highest rates of infection in 2011 were seen in Eastern Europe (22.4 per 100,000 population) followed by the West (6.5 per 100,000 population) and Central Europe (1.6 per 100,000 population)[139]. The highest rates of infection reported by individual countries were Ukraine (38.0 per

Figure 2.1.5 HIV infections, rates by geographical region 2004-2011[139]

Image not available due to copyright restrictions

100,000 population) followed by Estonia (27.3 per 100,000 population) and San Marino (25.6 per 100,000 population)[139]. Figure 2.1.6 shows the number of new HIV infections by country of Europe per 100,000 population reported in 2011.

Eastern Europe

In the UNAIDS estimates, Eastern Europe and Central Asia are grouped together because of their geographical location and similar population demographics[135]. The 2012 estimates show that this is the only region in Europe where the incidence of new HIV infections continues to rise (Figure 2.1.5)[135]. The 140,000 new HIV infections in this region means that there are now an estimated 1.4 million HIV-positive people living in Eastern Europe and Central Asia[135].

Since 2004, all countries that have consistently reported data have reported annual increases in the numbers of HIV diagnoses. A resurgence of HIV was reported by Latvia in 2007/08 and by Lithuania in 2009[139]. Among the other countries, rates have steadily increased since 2004, by more than three times in Armenia, Azerbaijan, Kyrgyzstan and Tajikistan, and more than twice in Georgia, Kazakhstan and Moldova. In Belarus and Ukraine increases of 59% and 76% were observed, respectively[139].

Figure 2.1.6 New HIV infections in Europe per 100,000 population in 2011[139]

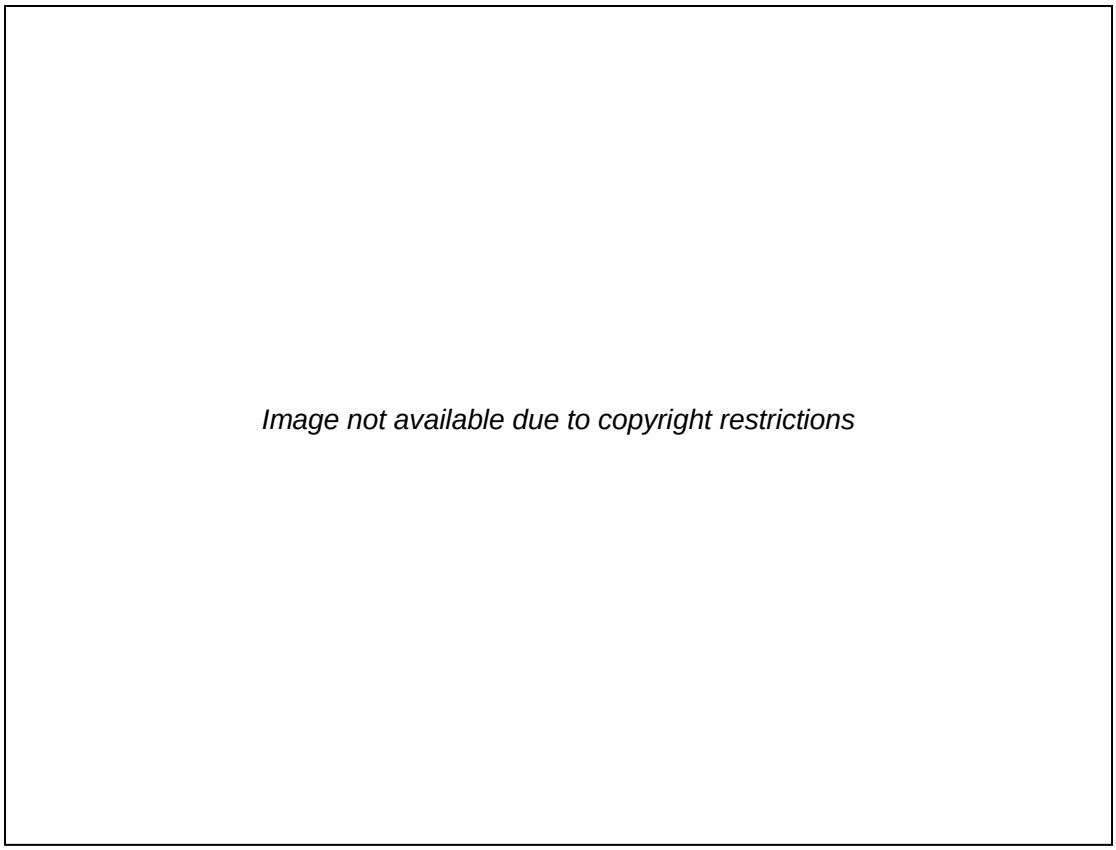


Figure 2.1.7 Trends of reported HIV diagnoses by transmission mode and year of diagnosis in Eastern Europe[139]

The overall number of HIV diagnoses among IDUs in Eastern Europe has increased by 24.7% since 2004, however, declines were reported in Belarus, Latvia, Lithuania and Moldova[139]. In contrast, there have been increases in IDU infection in Georgia, Kazakhstan, Kyrgyzstan, Tajikistan and Ukraine[139]. However, the predominant mode of HIV transmission in the East has changed to heterosexual contact in recent years. The overall number of HIV cases acquired by heterosexual contact increased 177.8% between 2004 and 2011, accounting for 56.7% of transmissions in 2011[139] (Figure 2.1.7). Further, increases in this mode of transmission have been reported by all Eastern countries except for Turkmenistan[139]. Although the number of HIV transmissions among MSM is relatively low in the East, the number of reported cases has increased eightfold, with all countries except Kyrgyzstan reporting an increase[139].

Western and Central Europe

In Western and Central Europe the total number of people living with HIV was estimated to be 900,000 in 2012, an increase of 41% from 2001[139]. The epidemic in the West is characterised by continuing increases in sexual transmission of HIV, particularly between men (Figure 2.1.8). Sex between men accounted for 40.1% of infections in the West in 2011, with 37.9% attributable to heterosexual sex and just 4.2% to IDU[139].

The epidemic in Central Europe remains low and stable, although there is evidence of increasing sexual transmission in many countries, especially between men but also increasingly among heterosexuals[139] (Figure 2.1.9). Men having sex with men accounted for 27.3% of HIV infections in 2011, followed by 25.7% due to heterosexual contact and 8.2% due to IDU[139].

Figure 2.1.8 Trends of reported HIV diagnoses by transmission mode and year of diagnosis in Western Europe[139]

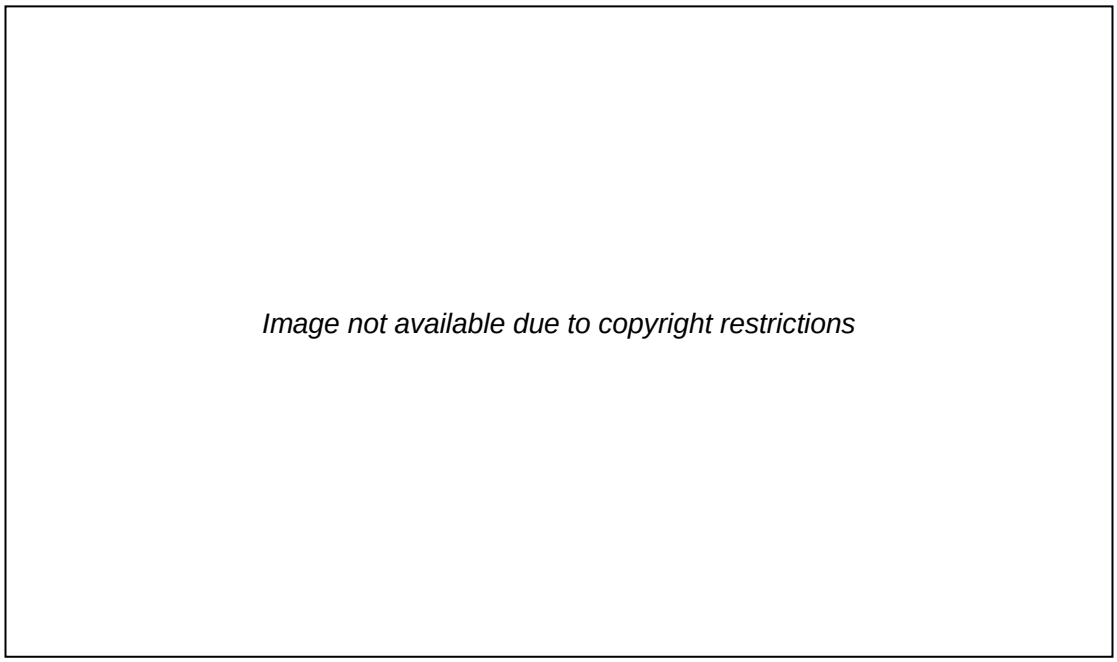
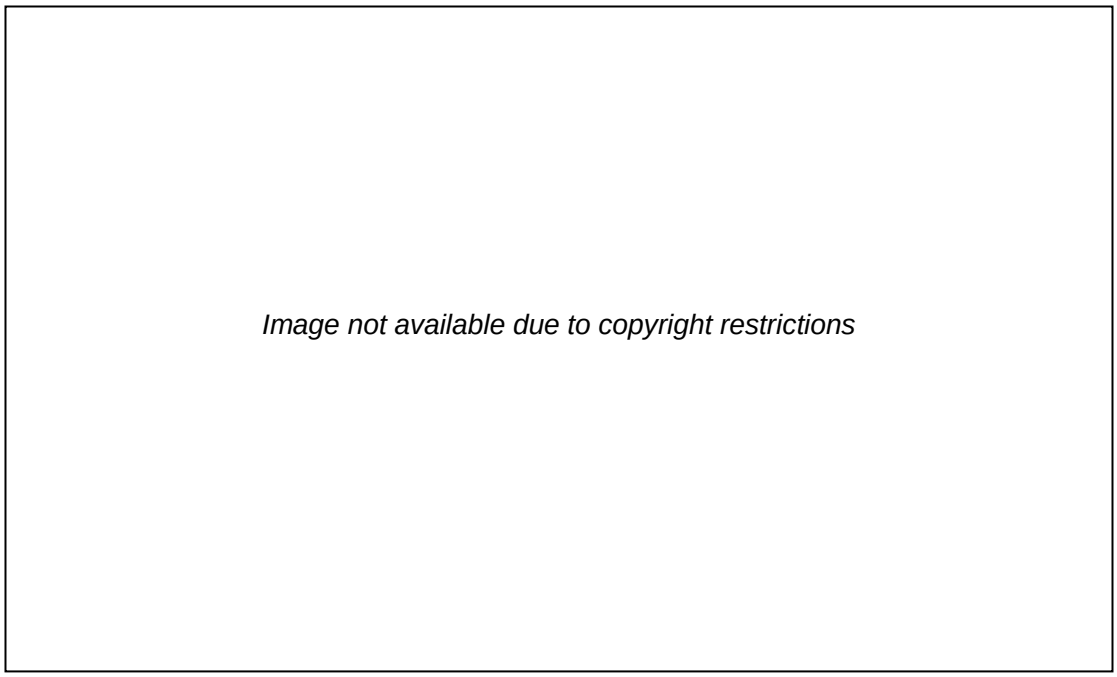


Figure 2.1.9 Trends of reported HIV diagnoses by transmission mode and year of diagnosis in Central Europe[139]



The heterogeneity of transmission also characterises the Central region, with different transmission modes predominating in different countries. In 2011, heterosexually acquired cases of HIV represented more than half of all diagnoses in Albania, Bosnia and Herzegovina, and Romania, while at least half of all cases of HIV were due to MSM contact in Croatia, Cyprus, Czech Republic, Hungry, Slovakia, and Slovenia[139].

2.1.8 Transmission of HIV

HIV is transmitted via contact with contaminated bodily fluids and the risk of infection at each exposure is heavily dependent on the amount of HIV virus circulating in the body[140;141]. HIV RNA has been found in many different bodily fluids, the most prominent being blood and semen, but it also exists in other fluids such as the tears of an infected person[29]. However, the levels of HIV RNA vary greatly by the location, whereas there may be significant levels of virus in blood and semen, even in an individual with a very high plasma viral load the amount of HIV RNA in a tear would be so small that it is impossible to be infected following contact with it[29]. Moreover, HIV is not able to survive outside of the body for prolonged periods, so infection via casual contact, shaking hands and kissing, or via public facilities, from the toilet seat, is not possible[29].

Consequently, HIV is most frequently transmitted during unprotected sex and via blood-to-blood contact during injecting drug use[142;143], while historically there was a major burden of HIV transmission via contaminated blood transfusions[144]. In the developing world, mother-to-child transmission is one of the major routes of infection as HIV RNA can be transmitted at birth and during the act of breastfeeding[145]. With the exception of mother-to-child and blood transfusion transmissions, the risk of infection from a one off exposure is usually low regardless of the transmission route, but in all cases repeated exposure will increase the risk of infection[146].

Sexual transmission

Worldwide, the most frequent route of HIV transmission occurs during unprotected sex[29]. However, the risk of infection during sexual intercourse depends on a number of factors, most importantly the level of HIV RNA in the blood[128]. Recent studies have shown that in discordant couples, where one partner is HIV-positive, the risk of the HIV-negative partner contracting HIV is approaching zero when the HIV-positive partner has undetectable HIV RNA due to continued successful treatment[147]. When HIV RNA is present the risk of infection will depend on the sexual acts performed, rough sexual practises such as fisting are likely to cause bleeding and are associated with transmission, the presence of other sexually transmitted infections and whether condoms were used[12;142;148].

During sex between a man and a woman, the woman is at greater risk of infection and worldwide approaching half of the entire HIV-positive population consists of women who were infected during sexual contact[12;149]. However, condoms have been shown to be a very effective method of preventing sexual transmission. In couples where one individual is HIV-positive, the risk of transmission of HIV when condoms are used correctly with no slipping

or splitting is negligible[12]. Unfortunately, in many settings gender inequality and financial constraints mean that women are unable to practise safe sex[149].

One intervention that has been found to reduce the risk of HIV transmission to men during sexual contact is circumcision, with risk reductions in the region of 60% reported among those circumcised[150-152]. Consequently, circumcision is recommended by the WHO to reduce HIV transmission in the developing world where access to condoms may be reduced or prevented by religious sentiment[153;154]. Unfortunately, circumcision infers no benefit for women in terms of HIV transmission, except that by reducing transmission to men there may be a smaller pool of HIV-positive men that could then transmit to women[155].

Sexual contact between men is the major transmission risk factor in the developed world, with receptive anal sex with an HIV-positive partner carrying the greatest risk[139;142]. The incidence of HIV infection among MSM has been rising in recent years, which is linked with an increase in unprotected sex evidenced by an increase in other sexually transmitted infections[135;156-159]. However, in some countries the rise in HIV incidence is no doubt linked to a better screening process and more frequent testing for HIV[160]. While sexual health and protecting partners from HIV infection is an important consideration for many HIV-positive MSM, there remains a section of the population who are either unaware of their infection status or ignore it and continue to take part in risky sexual practises[161-165].

Many interventions have been piloted among MSMs, with reduced transmission rates seen after the introduction of support groups at the individual and community level[166]. However, the incidence of HIV infection continues to rise in this population and it is thought that as treatment for HIV has become more and more successful, fear of HIV infection has decreased and risky sexual behaviour has increased[167].

Transmission via blood-to-blood contact

HIV is efficiently transmitted via blood-to-blood contact and many transmissions occur during injecting drug use when needles and other drug paraphernalia are shared[12;143;168]. The chance of contracting HIV during a year of engaging in IDU has been estimated to be up to 50% in some populations. Consequently, the number of HIV-positive IDUs continues to rise worldwide[169]. Interventions among the IDU community have focused on needle exchange programs, community outreach and education about the dangers of sharing equipment which can transmit blood[169]. In the UK and Australia these programs have helped to keep the rate of HIV infection low among IDUs[170;171]. However, other countries do not support such a liberal attitude to illicit drug abuse and IDUs are criminalised and

refused treatment for HIV, leading to very high rates of infection and HIV-related mortality[172-175].

Blood-to-blood transmission can occur in circumstances other than IDU. Professionals in the health service are at risk of accidental needle stick injuries or splashes onto open wounds or the eyes[12]. However, in these cases the risk of transmission is very low and has been estimated to be approximately 0.3% after an accidental needle stick and 0.09% after a splash of blood to the eye[176]. Further, treatment is available and widely used in these circumstances, known as post-exposure prophylaxis which is a short course of treatment that stops the HIV virus from taking hold and reduces the risk of transmission by as much as 90%[177].

Many people were also infected with HIV due to receiving infected blood products before the introduction of adequate screening mechanisms. Transmission from infected blood products has been shown to be very efficient, as infected blood is transfused directly to the blood stream, with transmission rates approaching 100%[144;178]. Thankfully, since the mid-1980s screening methods have been employed and all blood products in the United States, Canada and Europe have been screened for HIV[12]. The WHO also recommends procedures for ensuring blood safety, which are cost effective in the long term, however, many developing countries continue to fall short of the requirements for safe blood screening[179;180].

Mother-to-child transmission

Without effective treatment for both mother and child, transmission of HIV can occur in labour during birth and the act of breastfeeding[145;181-184]. Studies have shown that the HIV viral load of the mother at the time of labour is the most important predictor of perinatal transmission of HIV and that without treatment the risk of transmission to the child can be as high as 40%[145;185;186]. It is estimated that 330,000 (95% confidence interval (CI) 280,000-390,000) children acquired HIV in 2011, with almost all of these new infections due to mother-to -child transmission[135;187]. However, this represents a 43% decline since 2003 and a 24% decline from 2009 when 430,000 (370,000-490,000) transmissions were estimated to have occurred[135].

Mother-to-child transmission is an area where great strides have been made, timely diagnosis of HIV-positive mothers and appropriate treatment should result in transmission rates approaching zero[188]. International plans are in place to attempt to achieve the elimination of new HIV infections in children by 2015, while continued reductions in the number of transmissions per year mean that confidence is growing in the feasibility of these

ambitions[135]. However, while perinatal transmission in the developed world is already at low levels, this is not the case in the developing world where a large proportion of new infections each year are due to mother-to-child transmission[145].

In the developed world, testing of all pregnant women for HIV, appropriate treatment and the avoidance of breastfeeding has seen the number of mother-to-child transmissions as a proportion of all HIV transmissions fall drastically from approximately 25% to approaching zero[189-192]. In Europe, most pregnant mothers with HIV will be engaged in care prior to falling pregnant and will have been informed of the risks associated with stopping treatment during pregnancy[189;190]. Current guidelines suggest that pregnant women with HIV should be treated in the same fashion as non-pregnant women, with just one routinely used drug, Efavirenz, to be avoided[193].

Mother-to-child transmission in sub-Saharan Africa is common as the majority of HIV-positive people are women of child-bearing age[145]. Further, breastfeeding is common practice due to the cost of feeding infants with formula milk[145]. Consequently, it is estimated that 40% of mother-to-child transmissions in this region are a direct result of breastfeeding[191;194].

2.1.9 Treatment for HIV infection

2.1.9.1 The history of treatment

The history of drug development of ARVs for the treatment of HIV coincided with a period of change at regulatory bodies such as the FDA[195]. Pressure groups and activists led calls for change as potentially lifesaving drugs were being trialled but were not widely available for patients that required them[195]. Clinical trials with death as the primary study endpoint required too long for the benefits of treatment to become apparent[195]. In 1992 the FDA adopted new regulations whereby a standard was established for the approval of a drug based on its effect on a surrogate marker, and not a clinical outcome[196;197]. This paved the way for far shorter clinical trials of ARV drugs which focused on the ability to achieve an undetectable HIV viral load within a given timeframe (typically 24 or 48 weeks in modern trials)[198]. Also in 1992, the parallel track policy statement was made specifically with the intention of expanding the availability of investigational drugs for treatment of HIV[197]. These changes helped to extend treatment to a larger population and led to rapid development of new drugs.

At the beginning of the HIV epidemic, there were no effective treatments available for HIV infection. The first antiretroviral drug for HIV, zidovudine a nucleoside reverse transcriptase

inhibitor (NRTI), was approved for use in 1987[199]. Zidovudine was originally developed in the 1960s to combat cancers caused by as yet unknown human retroviruses[200;201]. The first clinical trial in HIV-positive people showed that zidovudine, AZT for short, was effective in reducing mortality and opportunistic infections, compared to a control arm receiving no active treatment[201]. Consequently, the trial was stopped early so that those in the control arm could also receive treatment with zidovudine[201]. However, there were severe adverse events associated with treatment, which are now known to have been mostly caused by early overdosing of the drug[202-205].

In the remainder of the decade, the effectiveness of zidovudine waned as resistance mutations began to develop in those taking the drug for prolonged periods of time, which meant that the benefits of treatment were limited to an extended life expectancy of up to 18 months[206-209]. Consequently, efforts were made to discover new drugs which could further extend the life expectancy of HIV-positive people and new drugs became available in the early 1990s[199;210].

In 1990, a new NRTI drug, didanosine (ddI), was evaluated in clinical trials with promising results documenting fewer adverse events than zidovudine, which lead to its approval by the FDA in 1991[211-213]. Shortly after, a similar formulation called zalcitabine (ddC) was also approved[199]. Switching to didanosine or zalcitabine from a failing zidovudine treatment often had substantial benefits to the patient[214]. This led investigators to study treatment regimens comprising of combinations of drugs, and it was soon found that taking either didanosine or zalcitabine along with zidovudine had a significant effect on progression to an AIDS-defining condition, compared to taking zidovudine alone[215]. However, the biggest strides towards effective treatment for HIV were taken when a new class of drug was developed in the mid-1990s, the protease inhibitor (PI)[199].

2.1.9.2 Combination antiretroviral therapy

Combination antiretroviral therapy (cART) involves combining multiple drugs from different classes, targeting different functions of the HIV lifecycle, into a single treatment regimen[201]. Introduced in 1995, cART is the gold standard of care for HIV-positive people worldwide[201]. In 1996, three PIs were approved for treatment of HIV, ritonavir, indinavir and saquinavir and cART consisted of two NRTIs, known as the cART backbone, and a PI[199;201]. Shortly after, in the same year a third class of drug was approved for treatment of HIV, the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine[199], which meant there were different combinations of cART available. Many studies have documented improved efficacy of cART in comparison to dual therapy, documenting improvements in CD4 cell count increases and faster HIV viral load suppression[216-222]. Combination antiretroviral

therapy has led to dramatic declines in HIV-related mortality, meaning that today HIV is seen as a chronic manageable condition[6;7]. Recent studies estimate that life expectancy of HIV-positive people in the developed world, established in care early during the course of infection, have life expectancy approaching that of those without HIV infection[8;9].

Originally, the PI indinavir was the most common third drug in a cART regimen, however it was not well tolerated due to complicated dose scheduling and severe renal toxicity[223]. Ritonavir was also found to be a potent PI, but with many associated toxicities that meant it was rarely prescribed at the full dose[224-228]. However, ritonavir was found to be a powerful inhibitor of PI metabolisation, and when administered in small doses along with other PIs acted as a booster to increase the bioavailability of other PIs without the associated side effects[229]. Consequently, most PIs are now prescribed along with a small dose of ritonavir, and these boosted PIs have been shown to be more efficacious than their unboosted counterparts[230-233].

As the number of antiretroviral drugs approved for treatment of HIV has increased over time, studies have tried to determine the most efficacious combinations with the fewest adverse effects and toxicities. Studies comparing PI-based cART with NNRTI-based cART have shown that NNRTI-based regimens have faster viral suppression of HIV RNA, but CD4 cell count responses are comparable regardless of the third drug class[234-237]. Differences between the combinations are only apparent with regards to the toxicity and resistance profile of the drugs, typically patients taking an NNRTI regimen may benefit from fewer toxicities while those taking PI based regimens will have a higher genetic barrier to resistance, so which regimen to use is often a choice made dependent upon the lifestyle and needs of the individual[238-242].

More recently, other types of drug have become available, namely integrase inhibitors which prevent the integration of HIV DNA into the host genome and fusion inhibitors which prevent the fusion between HIV and host CD4 cells[243]. As treatment has advanced to more potent less toxic drugs, attention has shifted from selecting treatment regimens based on efficacy alone, as most commonly used drugs now have comparable efficacy[244]. Government needs to reduce health sector spending have in some countries, the UK and Europe especially, led to cost effectiveness playing an increasing role in determining treatment guidelines[245;246]. New drugs are typically very expensive and may only offer marginal gains over older drugs, which can be much cheaper, especially those which have gone off patent[246].

2.1.9.3 Current treatment guidelines

Current treatment guidelines recommend that for adults first-line therapy should consist of two NRTIs plus a third drug from a different class, usually an NNRTI, PI or integrase inhibitor[1;247]. As efavirenz is available in a fixed-dose combination pill with two NRTIs, lamivudine and tenofovir, it is strongly recommended as the initial drug regimen to reduce the pill burden[247]. PIs are recommended as first-line therapy in children under the age of 3 and for those with anticipated poor adherence due to their higher genetic barrier to resistance mutations[1;247]. For children younger than 3 lopinavir boosted with ritonavir based regimens are recommended, while for adults the first-line PI is usually atazanavir, also boosted with ritonavir[1;247]. If there are contraindications to the use of either of the NRTIs then a different combination of NRTIs should be used, while if there are contraindications to efavirenz or it is not available the NNRTI nevirapine should be used[1;247]. Due to many different reasons there is still some debate over which choice of first-line treatment is optimal, one of the most important factors being the long term durability of a regimen.

Recommendations for when to start treatment are based on CD4 cell count and plasma HIV RNA viral loads and there is some debate over which are the optimal cut-offs in asymptomatic individuals. Current European guidelines recommend starting treatment once CD4 cell count drops below 350 cells/mm^3 [1], however, the most recent WHO recommendations have stated that treatment should be recommended for all patients with a CD4 cell count below 500 cell/mm^3 [247]. Early treatment decreases the risk of early CD4 cell depletion and lowers the viral load, which in turn, decreases the risk of infection to others[248]. Delaying therapy, on the other hand, avoids the risk of toxic drug effects and resistance mutation development and the reliance on new potent and tolerable drugs becoming available in the future[248]. The START (Strategic Timing of AntiRetroviral Therapy) study is a randomised controlled trial of ART-naïve patients with CD4 cell counts greater than 500 cell/mm^3, which aims to compare patients randomised to start therapy immediately with those that defer treatment until CD4 cell count declines to 350 cells/mm^3 [249]. The objective of this study is to help answer the question when is the optimal time to start treatment. Enrolment was completed in 2013 and three years of follow-up are expected[249].

2.1.9.4 Currently licensed antiretrovirals

Nucleoside reverse transcriptase inhibitors (NRTI)

NRTIs were the first class of drugs licensed for treatment of HIV[199]. NRTIs are nucleotide or nucleoside analogues needed for HIV replication, but with flaws inserted to interrupt the process. They attach to the HIV reverse transcriptase and stop HIV RNA from becoming

HIV DNA[250]. Therefore, when HIV's reverse transcriptase uses the faulty nucleoside analogue, rather than the natural nucleoside, the virus is unable to replicate[250]. Tables 2.1.3 and 2.1.4 summarise the NRTIs and NRTI fixed dose combinations currently licensed for the treatment of HIV in the U.S. and Europe. The currently recommended dual NRTI backbone of cART is the fixed-dose combination of emtricitabine and tenofovir[1;247].

Protease inhibitors (PI)

PIs were the second class of drug to become available and were the first used in combination with NRTIs to create combination therapy[199;251]. PIs interrupt the protease enzyme, which means that after HIV has replicated its genetic material the assembly of new HIV virons is inhibited, so that it may not go on to infect other cells[250]. Table 2.1.6 summarises the PIs currently licensed for HIV treatment in the U.S. and Europe. Atazanavir, darunavir and lopinavir, all boosted with ritonavir, are the currently recommended first-line PIs[1;247].

Table 2.1.3 NRTIs currently licensed for treatment of HIV

Generic drug name	***Trade name***	***EMA approved[252]***	***FDA approved[199]***
Abacavir (ABC)	Ziagen	08-Jul-99	17-Dec-98
Didanosine (ddI)	Videx	*	09-Oct-91
Emtricitabine (FTC)	Emtriva	24-Oct-03	02-Jul-03
Lamivudine (3TC)	Epivir	08-Aug-96	17-Nov-95
Stavudine (D4T)	Zerit	08-May-96	24-Jun-94
Tenofovir (TDF)	Viread	05-Feb-02	26-Oct-01
Zidovudine (AZT)	Retrovir	*	19-Mar-87

***Not licensed**

Table 2.1.4 Fixed dose NRTI combinations licensed for treatment of HIV

Generic drug name	***Trade name***	***EMA approved[252]***	***FDA approved[199]***
Emtricitabine/Tenofovir (FTC/TDF)	Truvada	21-Feb-05	02-Aug-04
Lamivudine/Abacavir (3TC/ABC)	Kivexa	17-Dec-04	02-Aug-04
Lamivudine/Abacavir/Zidovudine (3TC/ABC/AZT)	Trizivir	28-Dec-00	14-Nov-00
Lamivudine/Zidovudine (3TC/AZT)	Combivir	18-Mar-98	27-Sep-97

Table 2.1.5 PIs currently licensed for treatment of HIV

Generic drug name	*Trade name*	*EMA approved*[252]	*FDA approved*[199]
Atazanavir/r (ATV/r)[1]	Reyataz	02-Mar-04	20-Jun-03
Darunavir (DRV/r)	Prezista	16-Dec-08	23-Jun-06
Fosamprenavir/r (FPV/r)	Telzir	12-Jul-04	20-Oct-03
Indinavir (IND)	Crixivan	04-Oct-96	13-Mar-96
Lopinavir/r (LPV/r)	Kaletra	20-Mar-01	15-Sep-00
Nelfinavir (NFV/r)	Viracept	*	14-Mar-97
Ritonavir	Norvir	26-Aug-96	01-Mar-96
Saquinavir/r (SQV/r)	Invirase	04-Oct-96	06-Dec-95
Tipranavir (TPV/r)	Aptivus	25-Oct-05	22-Jun-05

/r: boosted with ritonavir; [1]Can also be taken without ritonavir; *Not licensed

Non-nucleoside reverse transcriptase inhibitors (NNRTI)

Like NRTIs, NNRTIs prevent the conversion of HIV RNA into DNA. NNRTIs connect to the HIV reverse transcriptase and change its shape, meaning that it no longer fits to the HIV RNA[250]. As a result the RNA cannot be converted into DNA. Table 2.1.5 summarises the NNRTIs currently licensed for treatment of HIV in the U.S and Europe. Efavirenz, nevirapine and rilpivirine are the recommended first-line NNRTIs[1;247]. NNRTIs also form part of single pill regimens which are fixed-dose combinations of two NRTIs and an NNRTI. Atripla, which combines efavirenz with truvada, is currently the recommended first line regimen according the WHO[247]. Table 2.1.7 summarises the fixed-dose single pill regimens currently licensed for HIV treatment.

Table 2.1.6 NNRTIs currently licensed for treatment of HIV

Generic drug name	*Trade name*	*EMA approved*[252]	*FDA approved*[199]
Efavirenz (EFV)	Sustiva	28-May-99	17-Sep-98
Etravirine (ETV)	Intelence	28-Aug-08	18-Jan-08
Delavirdine (DLV)	Rescriptor	*	04-Apr-97
Nevirapine (NVP)	Viramune	05-Feb-98	21-Jun-96
Rilpivirine (RPV)	Edurant	28-Nov-11	20-May-11

***Not licensed**

Integrase/fusion/entry inhibitors

Table 2.1.8 summarises the other antiretrovirals currently licensed for HIV treatment. Maraviroc is an entry inhibitor which blocks the chemokine co-receptor 5 (CCR5) which HIV uses to bind to and enter CD4 cells[250]. It is the first example of an anti-HIV drug that blocks

Table 2.1.7 Single pill regimens currently licensed for treatment of HIV

Generic drug name	*Trade name*	*EMA approved*[252]	*FDA approved*[199]
Efavirenz/Emtricitabine/Tenofovir (EFV/FTC/TDF)	Atripla	13-Dec-07	12-Jul-06
Rilpivirine/Emtricitabine/Tenofovir (RPV/FTC/TDF)	Eviplera	28-Nov-11	10-Aug-11

cellular function rather than viral function, but its use is limited to HIV virus types that use the CCR5 receptor. Enfurvitide is a fusion inhibitor that prevents HIV from joining with and infecting healthy CD4 cells[250], however, it is expensive and has to be injected twice daily, so often it is reserved for patients who have exhausted all other treatment options[247]. Raltegravir and dolutegravir are integrase inhibitors which interfere with the integrase enzyme responsible for integrating HIV DNA with the host DNA. Without this process HIV cannot use the host cells natural process to replicate its genetic material[250].

Table 2.1.8 Integrase/fusion/entry inhibitors licensed for treatment of HIV

Generic drug name	*Trade name*	*EMA approved*[252]	*FDA approved*[199]
Maraviroc	Celsentri	18-Sep-07	06-Aug-07
Enfuvirtide	Fuzeon	27-May-03	13-Mar-03
Raltegravir	Isentress	20-Dec-07	12-Oct-07
Dolutegravir	Tivicay	16-Jan-14	12-Aug-13

2.1.9.5 Limitations of cART

Although cART has transformed HIV from a deadly disease to a chronic manageable infection, cART cannot cure HIV[6]. It works by inhibiting HIV while it attempts to integrate with the host's CD4 cells and reproduce. However, HIV remains in reservoirs in the body that cART cannot reach[97]. Once cART is stopped HIV will begin to reproduce again and without treatment will lead to death[97]. Therefore, treatment for HIV remains a life-long commitment. Further, some patients will fail treatment due to the development of resistance, poor adherence to treatment will lead to lower levels of drug in the body and the chance for HIV to develop resistance[253;254], while others will stop treatment due to unmanageable side effects and toxicities.

ARV resistance mutations in HIV can be acquired during transmission, where a mutated HIV virus is directly transferred to another, or they may develop during the course of ARV treatment[255]. If an individual taking ART has suboptimal adherence then complete suppression of the virus may not occur. When this is the case the remaining unsuppressed

HIV virus will adapt to tolerate the presence of ART, meaning that those drugs can no longer suppress the virus[255]. Consequently, use of those drugs, even with optimal adherence, may not be enough to control HIV infection and will lead to treatment failure and the need to switch to a different ARV drug or drug class[255].

Toxicities and adverse events associated with ARVs

As with all medications, patients taking ARVs often experience adverse events. Although ARV-related drug toxicity has decreased as newer drugs have become available, there remains a significant burden of drug-related toxicity associated with treatment with ART[256]. Some NRTIs, particularly the older drugs, are known to inhibit mitochondrial activity within cells, which in the long term can lead to serious side effects[256-258]. These include myopathy (zidovudine), neuropathy (stavudine, didanosine, zalcitabine), hepatic steatosis and lactic acidosis (didanosine, zidovudine, stavudine) and lipoatrophy and lipohypertrophy (all NRTIs but particularly stavudine), some of which can be fatal if treatment is not discontinued[256-258].

NNRTIs are commonly associated with rash and lipid disorders[257]. The rash can be so severe that it will require treatment discontinuation and use of different ARV drug classes, whereas lipid disorders can often be managed with the use of statins and other supplements[257;258]. Nevirapine and efavirenz are both first generation NNRTIs that commonly prescribed, however, nevirapine is associated with severe hepatotoxicity while up to 50% of patients taking efavirenz can suffer from central nervous system adverse effects during the first months of treatment[257;258]. Users of the second generation NNRTI etravirine also experience rash (in 20% of patients) which indicates discontinuing treatment[257]. Further, etravirine is metabolised in the liver by similar processes to other drugs leading to significant drug-drug interactions[257;258].

PIs are also extensively metabolised in the liver and are associated with the most drug-drug interactions[257]. Common adverse effects of PIs include gastrointestinal effects, lipohypertrophy, glucose intolerance or diabetes mellitus, and lipid disorders[256-258]. Approximately 60% of patients taking PIs have elevated total cholesterol levels and over 75% have high triglyceride levels[257]. Atazanavir without ritonavir boosting is considered the most lipid-friendly PI, followed by boosted darunavir and boosted atazanavir[257]. A full list of ARV drugs and their most commonly associated toxicities are shown in Table 2.1.9.

Table 2.1.9 Toxicities and adverse effects associated with ARV drugs[1;256-258]

Drug class	*Generic name*	*Toxicities/adverse effects*
NRTI	Lamivudine	Nausea, vomiting, diarrhoea, headache, abdominal pain, hair loss, fever, insomnia, rash, tiredness, joint pain, lactic acidosis (rare), liver damage (rare)
	Emtricitabine	Nausea, diarrhoea, headache, raised creatine kinase levels, skin darkening, lactic acidosis (rare), liver damage (rare)
	Zidovudine	Nausea, vomiting, fatigue, headache, dizziness, weakness, muscle pain, loss of appetite, fever, dyslipidaemia, blood disorders (rare), lipoatrophy (rare), lactic acidosis (rare)
	Abacavir	Nausea, vomiting, diarrhoea, fever, headache, abdominal pain, tiredness, loss of appetite, hypersensitivity reaction (rare), lactic acidosis (rare)
	Tenofovir	Nausea, vomiting, diarrhoea, flatulence, dizziness, weakness, rash, headache, stomach pain, fatigue, bloating, kidney problems (rare), bone thinning (rare)
	Stavudine	Nausea, vomiting, fatigue, headache, dizziness, weakness, rash, itching, heartburn, steatosis, lipoatrophy, lipohypertrophy, peripheral neuropathy, dyslipidaemia, pancreatitis (rare), lactic acidosis (rare)
	Didanosine	Nausea, vomiting, diarrhoea, abdominal pain, rash headache, peripheral neuropathy, pancreatitis (rare), lactic acidosis (rare)
NNRTI	Efavirenz	Rash, dizziness, sleep disturbance, abnormal dreams, impaired concentration, nausea, vomiting, headache, tiredness, diarrhoea, anxiety, depression, psychosis (rare), liver problems (rare)
	Etravirine	Rash, peripheral neuropathy, severe rash (rare), Steven Johnson syndrome (rare)
	Nevirapine	Liver toxicity, allergic reaction, rash nausea, headache, fatigue, stomach pain, diarrhoea, severe rash (rare), Steven Johnson syndrome (rare)
	Rilpivirine	Insomnia, headache, rash, raised liver enzymes, depression, dizziness, stomach pain, vomiting
PI	Atazanavir	Nausea, diarrhoea, rash, stomach ache, headache, insomnia, vomiting, hyperbillirubinaemia, lipodystrophy, liver toxicity, diabetes, kidney stones (rare), changes in heart rhythm (rare)

Drug class	*Generic name*	*Toxicities/adverse effects*
	Darunavir	Diarrhoea, nausea, rash, stomach pain, vomiting, headache, lipodystrophy, liver toxicity, diabetes, fever, abnormal liver function (rare), changes to heart rhythm (rare)
	Lopinavir	Lipodystrophy, raised liver enzymes, nausea, vomiting, diarrhoea, abdominal pain, weakness, heartburn, headache, raised lipids, liver toxicity, diabetes
	Ritonavir	Raised lipids and liver enzymes, nausea, vomiting, diarrhoea, abdominal pain, headache, weakness, bad taste in the mouth, lipodystrophy, liver toxicity, diabetes
	Tipranavir	Nausea, diarrhoea, vomiting, abdominal pain, tiredness, headache, fever, liver abnormalities, rash, lipodystrophy, liver toxicity, diabetes, flatulence
CCR5 inhibitor	Maraviroc	Nausea, diarrhoea, fatigue, headache, allergic reaction (rare), liver problems (rare)
Integrase inhibitor	Raltegravir	Headache, insomnia, severe rash (rare), hypersensitivity reaction (rare) extreme thirst (rare)
	Dolutegravir	Headache, insomnia, lipodystrophy (rare), ALT/AST elevation in hepatitis B or C coinfection individuals

2.2 Hepatitis

Hepatitis is a medical condition that causes inflammation of the liver and is characterised by the presence of inflamed liver cells[259]. It can be a self-limiting condition that heals on its own or it can progress to scarring, fibrosis, or cirrhosis of the liver[259]. Hepatitis sufferers often present with symptoms of jaundice, anorexia and general malaise, although occasionally sufferers report limited or no symptoms[259]. Most cases of hepatitis are caused by a group of viruses called the hepatitis viruses, although it can be caused by other infections, certain medications and alcohol use[259].

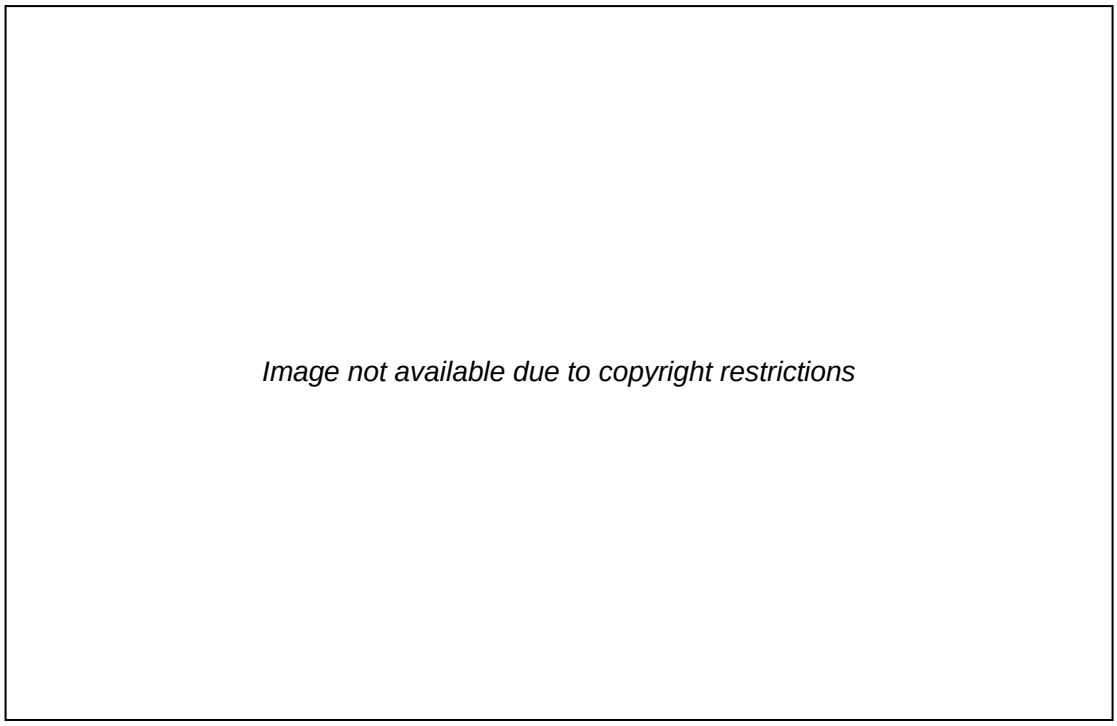
In the middle of the 20th century viral hepatitis was believed to consist of two major types only – infectious or type A hepatitis and serum or type B hepatitis[260;261]. Cases were typically recognised through symptoms such as jaundice and in the absence of diagnostic assays, the two types were distinguished based on the circumstances of exposure, the faecal-oral route and a short incubation period for hepatitis A (typically 15 – 50 days) and blood exposure and a long incubation period for hepatitis B (typically 30 – 180 days)[260]. The introduction of blood donor screening for hepatitis B reduced post-transfusion hepatitis by just 25%[261], while transfusion studies identified an 'acute hepatitis' that straddled the incubation periods of types A and B and often lacked typical hepatitis-like symptoms and jaundice[260], inferring the existence of a third hepatitis virus initially referred to as non-A non-B hepatitis. Although in 1974 non-A non-B hepatitis was briefly referred to as hepatitis C[262], it wasn't until 1989 with the development of specific serological tests that non-A non-B hepatitis and hepatitis C were confirmed to be one and the same[263-265].

2.2.1 Hepatitis C Virus

Hepatitis C virus (HCV) is a small-enveloped positive-strand RNA virus that has been classified in the genus, *Hepacivirus*, within the family *Flaviviridae*[266-268]. HCV, as shown in Figure 2.2.1, consists of a core of genetic material (RNA) surrounded by a capsid shell of protein and further encased in a lipid envelope of cellular origin, in which two viral envelope glycoproteins, E1 and E2 are inserted[266].

Since it was discovered in 1989, HCV has been recognised as a major cause of chronic liver disease worldwide[269]. The World Health Organisation's (WHO) most recent estimate puts the worldwide number of people chronically infected with HCV at between 130 and 170 million. An estimated 3 to 4 million people become newly infected each year, while 350 thousand people are estimated to die each year from HCV liver-related diseases[270]. Though the HCV endemic is worldwide, the prevalence varies substantially by WHO

Figure 2.2.1 Structure of the hepatitis C virus[271]



geographic region[269]. HCV is predominantly spread by contact with infected blood and the epidemic has thrived in areas with poor or no control over blood products as well as high prevalence of injecting drug use[272]. As shown in Figure 2.2.2, the highest reported prevalence rates are in Africa and Asia, while Western Europe and the Americas have lower prevalence rates. More specifically, in Europe there appear to be 3 distinct areas of HCV prevalence[273]. In Northern European countries the prevalence ranges from 0.1% to 1.0%[269;273;274], in Central European countries the prevalence is deemed intermediate ranging from 0.2% in the Netherlands to 1.2% in France[273;275;276]. In Southern European countries the overall prevalence ranges from 2.5% to 3.5%[273;276-278]. Epidemiological data from Eastern Europe is scarce but the prevalence of HCV is believed to be far higher, ranging from 1% in among blood donors up to 90% in high risk groups[273]. A breakdown of the prevalence of HCV in Europe can be seen in Figure 2.2.3.

2.2.2 Transmission and Risk Groups

HCV is a blood-borne infection and as such is primarily transmitted via the parenteral route of direct blood-to-blood contact[279;280]. As such the rapid spread and worldwide dissemination of HCV closely mirrors the invention and spread of blood transfusion methods, blood products and other invasive medical procedures that became increasingly available during the 20th century[281]. There has, however, been much debate about potential modes of transmission of HCV. It was previously thought that only about half of the reported cases of acute HCV have a defined parenteral exposure[282], though with evidence of other routes of transmission now established it's now believed that in only 10% of cases

Figure 2.2.2 Geographic variability in the prevalence of HCV[269]

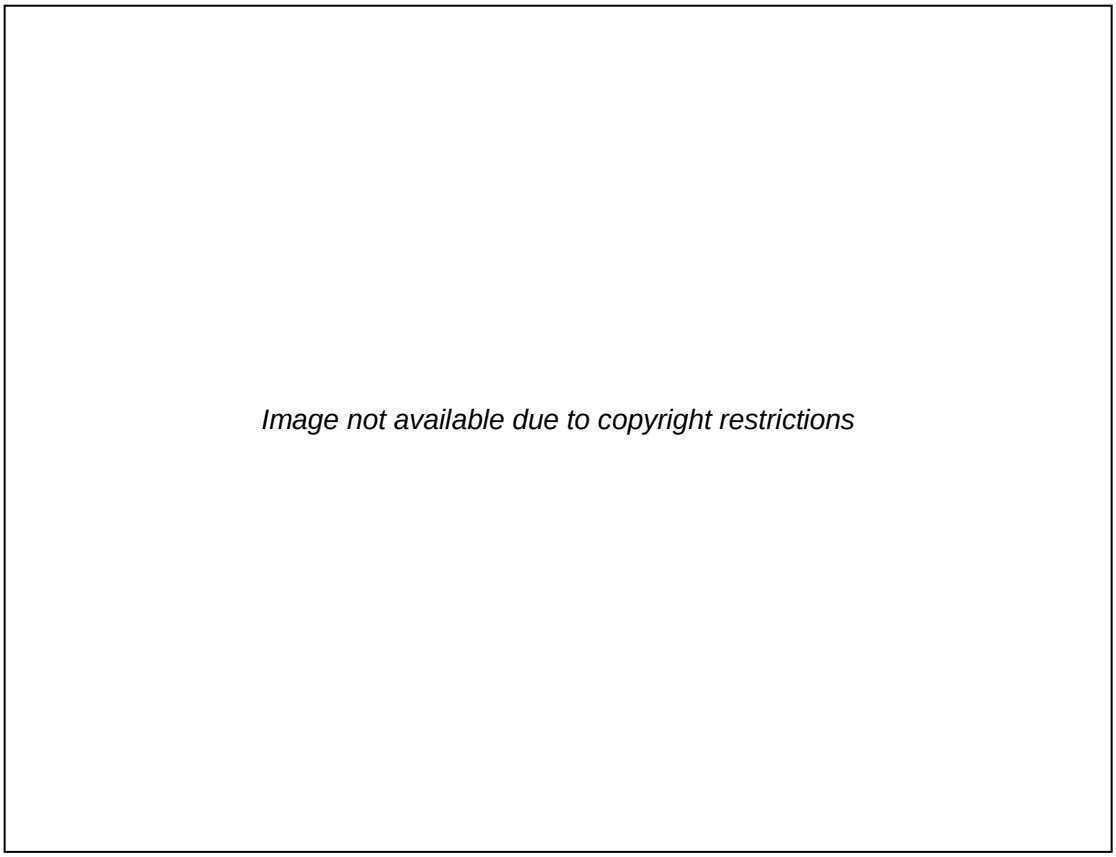
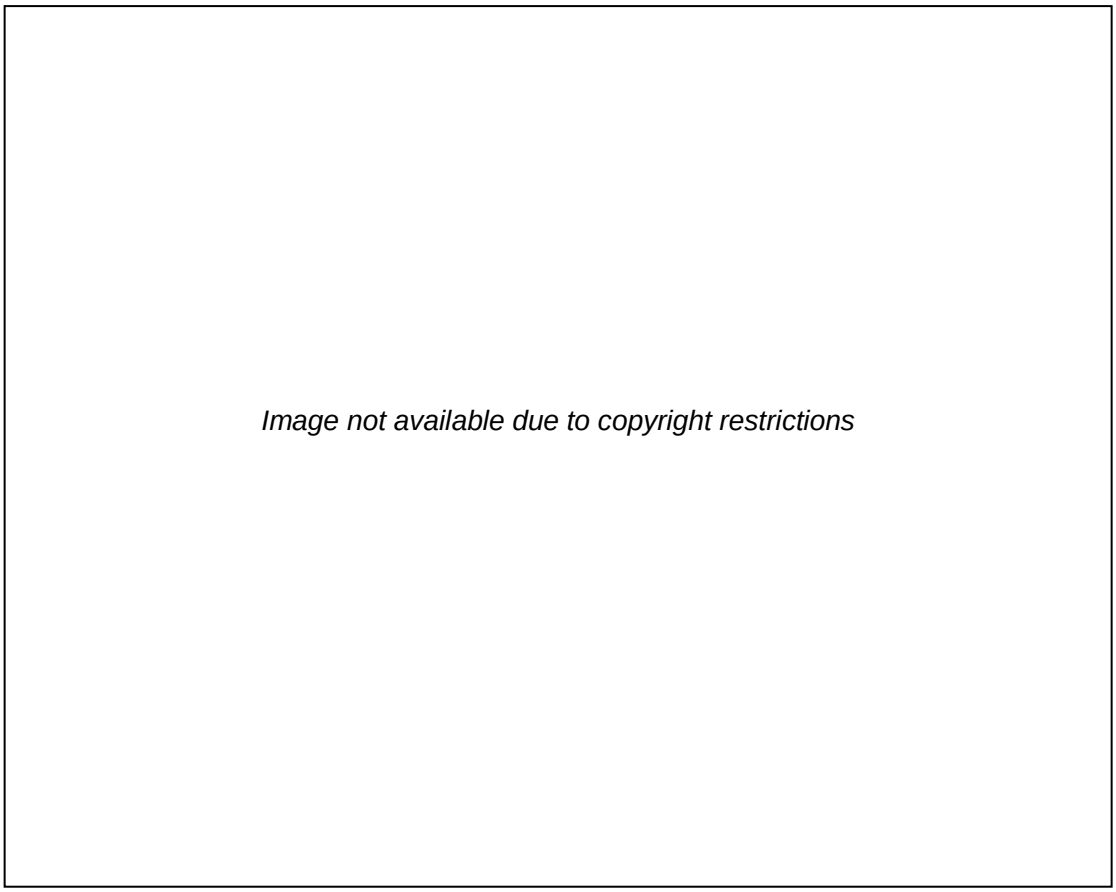


Figure 2.2.3 European HCV prevalence[273]



the route of HCV transmission is unknown[283], which is often described as 'community acquired' HCV[280].

2.2.2.1 Injecting drug use

In resource rich countries the predominant mode of HCV transmission is needle sharing in IDUs, which accounts for over 50% of HCV-infected patients[284]. Studies from different continents have shown prevalence rates of HCV in IDUs of between 50% and 90%, while the strongest predictor of transmission appears to be the duration of IDU[285-287]. However, in recent times with focus being given to safe needle programs since the onset of the HIV epidemic, transmission of HCV by the sharing of drug preparation equipment may have become more important[288]. Studies have shown that sharing 'cookers' (used to heat and mix the drugs) and cotton filters (used to strain out particles as the drug is drawn up into the syringe) may cause transmission[288;289]. Further, higher transmission rates have also been shown in those sharing straws used for snorting cocaine[284;290].

2.2.2.2 Blood transfusion

Haemophiliacs and others requiring blood transfusions are also at risk of HCV transmission. Before the initiation of HCV antibody screening it's estimated that approximately 5% of individuals who had received blood transfusions or other blood products had seroconverted to anti-HCV positive, with a relative risk of 0.45% per unit transfused[280;291]. Further, haemophiliacs who receive clotting factors pooled from a number of donors were at a far higher risk of infection, with 10%-20% seroconverting[280]. Clinicians in developed countries are now dealing with the long-term effects of past epidemics of transfusion-associated HCV. Adult cohort studies have estimated that at an average of 15 years after contaminated blood transfusion 75% of people are HCV RNA positive and that the frequency of liver cirrhosis is 20%[292-294]. However, since September 1991 when screening of blood donors was introduced, the risk of acquiring HCV through a blood transfusion in resource-rich countries has been extremely low, with the risk of contracting HCV from a blood transfusion in the UK now estimated to be 1 in 2,000,000[295;296]. The residual risk is a consequence of the short window period whereby a newly infected person does not yet have HCV antibodies[295]. Most people that are infected with HCV will have HCV antibodies within 5-10 weeks and with the introduction of HCV nucleic acid testing the potential window period for missing an infection is down to 17 days[295;297].

The situation is, however, very different in developing countries where blood transfusion remains a major cause of the spread of HCV. Between 2001 and 2002 it is estimated that 6 million blood units were not screened for major blood-borne infections, though this a considerable reduction from the period between 1998 and 1999, it cannot be considered

sufficient to limit the HCV epidemic in these areas[281]. The WHO blood safety report in 2011 states that 39 countries still do not routinely test for transfusion-transmissible infections, while 47% of blood donations in low-income countries are tested in laboratories without quality assurance[270].

2.2.2.3 Mother to child transmission

Transmission of HCV can also take place vertically from mother to child perinatally, and although comparatively rare, it is considered the most important mode of transmission in childhood acquisition due to the improvements in screening methods for HCV in blood donors and the foetal toxicity of currently available medications to treat HCV[298]. HCV viral load is an important factor in determining the chance of transmission from mother-to-child with many studies indicating that higher HCV RNA levels were related to increased risk of transmission[299;300]. In women with positive HCV RNA the risk of transmission has been reported as 4% to 7% per pregnancy[298], but this has been shown to be far higher in those coinfected with HIV with 4- to 5-fold increases in transmission being reported in some studies[286;298;301]. It's unclear whether the method of delivery is associated with transmission of HCV as some studies suggest that transmission is more likely to occur with vaginal delivery[302;303], while other studies do not confirm this finding[304;305]. Despite the fact that HCV has been found in breast milk, breastfeeding is not thought to be a significant risk of transmission as long as the mother's nipples are not cracked or bleeding[298].

2.2.2.4 Needle stick injuries

As HCV can be transmitted by contaminated needles transmission of HCV to or from health-care workers from needle stick or mucous membrane splash injuries are well documented[295;306;307]. However, it seems that HCV is not transmitted efficiently through occupational exposures to blood as it has been estimated that the risk of transmission from a single percutaneous exposure from an HCV-positive source is 1.8% (range 0% to 7%)[308]. More recently tattooing has also been considered as a potential route of HCV transmission. Most studies agree that tattooing is an independent risk factor for HCV[279], while a study from the USA in the early 1990s found those who had tattoos acquired in commercial tattoo parlours to have a risk of chronic asymptomatic HCV[309]. Due to the nature of tattooing and the comparatively small amount of HCV entering the body sub-dermally via contaminated needles, it's thought that tattooing is less likely to cause acute infection but chronic asymptomatic HCV[285]. However, with the impact of HIV on the use of safe needles and awareness of safe practises, the Center for Disease Control and Prevention (CDC) now believe the risk of acquiring HCV from a licensed tattoo parlour to be negligible[310]. It is also possible for HCV to be transmitted via household contacts. Studies have shown that sharing razor blades can be considered a risk factor for transmission[311], while it's also been

shown that toothbrushes of HCV-positive people can harbour detectible amounts of HCV RNA[312].

2.2.2.5 Sexual transmission

The frequency of HCV transmission via sexual contact is an issue of contention among medical professionals[313]. The potential for sexual transmission is demonstrated by the fact that HCV RNA has been found to be present in semen and vaginal secretions[314;315], however, the likelihood of transmission appears to depend on many factors and HCV seems to be less efficient at transmitting sexually than other blood-borne infections such as hepatitis B[280]. In heterosexual couples the risk of transmission appears to be minimal. Several large cohort studies have failed to show an increased risk in HCV transmission among discordant couples[316;317]. With long follow-up periods of over 10 years in these studies it was estimated that the probability of transmission in heterosexual couples is as low as 1 in 10 million sex contacts[316]. Some studies have shown the presence of the same virus in couples by molecular analysis[318;319], but these studies have been unable to rule out other common exposure routes.

A potential confounder in studies dealing with sexual HCV transmission in heterosexual couples is the duration of the relationship. A few studies have shown an increased risk of transmission in couples in longer relationships[320;321], but other larger studies that controlled for age did not confirm this finding[322-324]. One possible explanation for this is that couples who have been together for longer have more time in which to be exposed to other common routes of transmission[313]. Compared to couples in regular relationships, people having multiple sexual partners are at a higher risk of acquiring HCV through heterosexual contacts[325]. One study showed that women having sexual contacts with 2 to 4 partners were nearly 3 times more likely to acquire HCV than those with 1 steady partner[326]. It seems that data regarding sexual transmission of HCV should be treated with some degree of trepidation however, as Italian studies have suggested that transmissions between heterosexual couples could be explained by the common practise of sharing needles or other known transmission routes[327;328].

Another risk factor for transmission between heterosexual couples in the pre-existence of sexually transmitted infections (STIs), with 1 study showing a 3-fold increased risk of transmission to an individual with an STI than without[329]. In particular individuals with HIV are at a higher risk of acquiring HCV through heterosexual contact, especially among those partaking in high-risk sexual behaviour having unprotected sex with multiple partners[330;331]. In a large study of women that controlled for intravenous drug use (IDU), HIV-positive women were twice as likely to acquire HCV[331]. Similarly a cross-sectional study in

Baltimore, USA demonstrated a 4-fold increase in the risk of transmission of HCV in HIV-positive individuals compared with those who were HIV-negative[329].

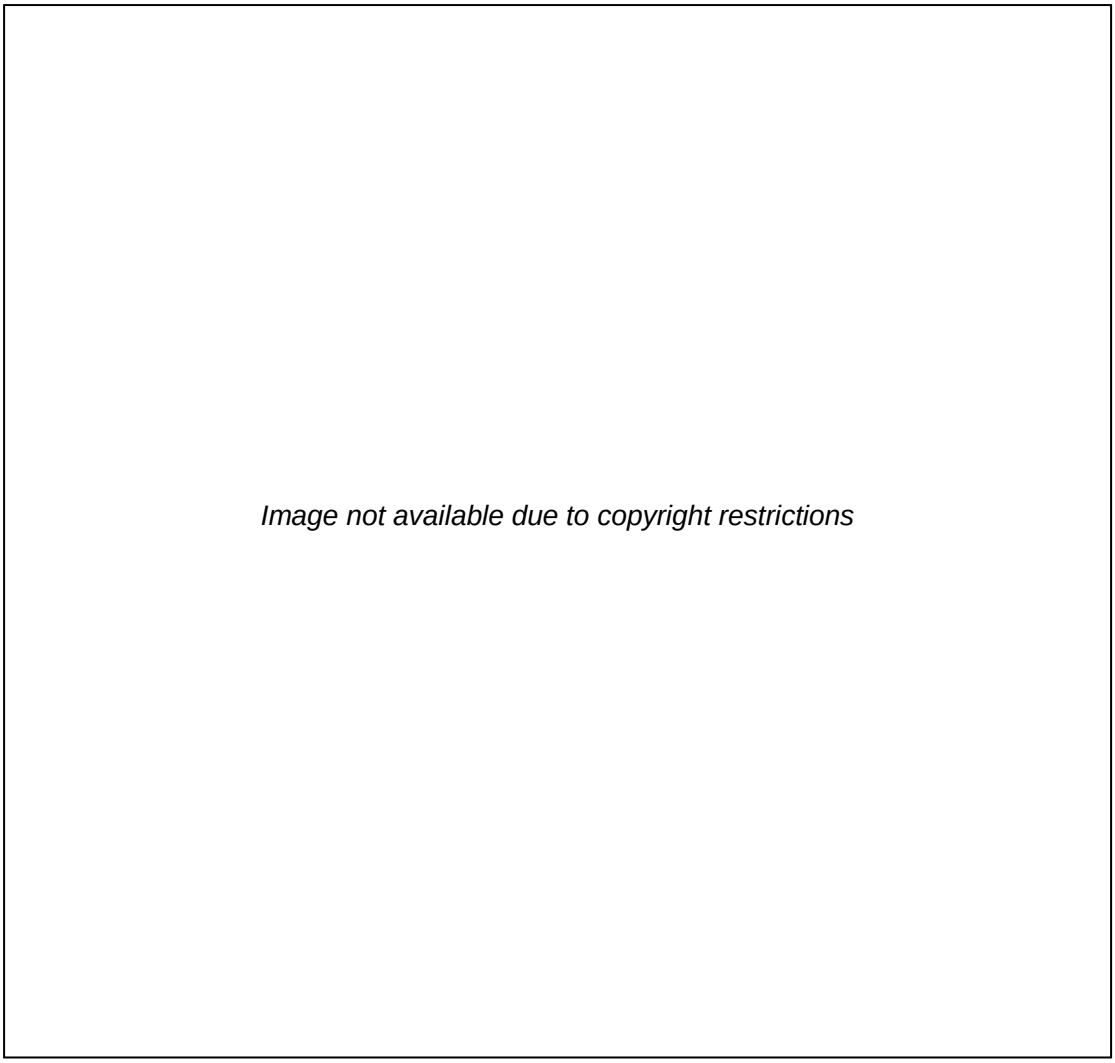
The risk of sexual transmission of HCV in HIV-negative men who have sex with men (MSM), although comparatively higher than heterosexuals, remains low. Infection rates have varied from 0 cases in 100 person-years in Amsterdam[332] to 1.5 cases per 1,000 person-years in the UK[333]. The situation in HIV-positive MSM is far more serious, especially in those who engage in high-risk and traumatic sex practises[313]. It has been estimated that the incidence of acute HCV infections among HIV-positive MSM in the UK has increased by 20% per year since 2002[334;335]. In a French cohort study the incidence increased 1.2 cases per 1,000 person-years before 2003 to 8.3 cases per 1,000 person-years after 2003[336]. Many longitudinal studies have also looked at the risk of transmission by HIV serostatus and found that HIV-positive MSM had between 4.1- and 5.7-fold higher odds of acquiring HCV than HIV-negative MSM[332;333;337]. The main reasons for this increased risk in HIV-positive MSM appear to be engaging in unsafe sexual practise and selection of sexual partners. Recent studies have reported on the practice of “serosorting” among HIV-positive MSM, whereby partners aware of their HIV-positive status engage in unprotected sex potentially unaware that they are coinfected with HCV[338;339]. HCV transmission has also been linked to sex with multiple partners[340;341], the use of sex toys and participation in group sex[338], which can potentially lead to mucosal damage[342]. Because of this researchers have surmised that the true risk of HCV sexual transmission in HIV-positive people comes down to blood to blood contact during sex[343].

2.2.3 Viral Replication

Due to the difficultly in detecting and identifying the virus (Section 2.2.3), it was originally thought that HCV replicated poorly *in vivo*[344]. However, it has since been discovered that this is not the case and that HCV infection is a highly dynamic process with a viral half-life of approximately 3 hours and up to 10^{12} virons produced per day in an infected individual, which is about 100 times greater than the rate reported for HIV[344-346]. HCV only infects humans and chimpanzees and due to the lack of a convenient animal model, knowledge of the molecular mechanisms of HCV replication is based primarily on analogies to the closely related flavi- and pestiviruses[347;348]. The current idealised HCV life cycle can be seen in Figure 2.2.4.

Once in the body the first step of the virus life cycle is the attachment of the infectious particle to a host cell. HCV predominantly targets hepatocytes but infection of B cells, dendritic cells and other cell types has been reported[348]. To attach to a host cell a

Figure 2.2.4 HCV life cycle[344]



protein on the surface of the viron particle must interact with a receptor on the host cell. Recently, CD81 a tetraspanin protein that is found on the surface of many cell types has been identified as a receptor for HCV due to its strong interaction with the E2 glycoprotein[349]. However, whether the virus binding to CD81 receptors is followed by internalisation of the virus particle is not yet understood. Apart from this route, HCV can enter the host cell by binding to low-density lipoprotein receptors[347]. When the virus particle enters the host cell it is uncoated and the RNA strand genome is liberated, a process not currently well understood. Unlike HIV, HCV is not integrated into the host genome, but fulfils 3 main roles, first as a messenger RNA for translation of the viral proteins, second as a template for the RNA to replicate, third as a newly generated genome to be packaged within new HCV particles ready to be released[344;347;348;350].

2.2.4 Genetic Variability

Genetic variability exists at several different levels in HCV. Due to the lack of a proof-reading function in the RNA replication it's estimated that each time HCV replicates there is

a 25% chance of an error occurring resulting in genetic variantion[351-353]. Coupling this with the rate of virus production of up to 10^{12} virons per day produces a highly genetically diverse population[354].

The most obvious variability is found in the divergence of the genotypes and subtypes of HCV which often reflect different geographical populations and transmission risk groups[354]. Nucleotide sequencing has revealed 6 major genetic groups, which on average differ from each other by 30%-35%. Within each genotype there exist more closely related subtypes that typically differ by 20%-25% from each other, categorised as 1a, 1b, 2a, 2b and so on, the most commonly found of which are displayed in Figure 2.2.5[346;354;355].

The genotypes most commonly encountered in the clinical setting are those distributed widely as a result of transmission via blood transfusion and needle sharing between IDUs predominantly in Western countries, namely 1a, 1b and 3a[354;355]. However, the variability in the HCV genome found in parts of Africa and South East Asia is rather different, where there appears to be close association between genotype and specific geographical region. Infections in Western Africa are predominantly HCV genotype 2[356-358], whereas those in Central Africa are caused by genotypes 1 and 4[359-361]. Similarly, in South East Asia infections are usually caused by genotypes 3 and 6 as shown in Figure 2.2.6[357;362;363].

In these regions the diversity of subtypes found within genotypes has been extraordinary, in a study of 23 HCV positive individuals in Ghana, 20 were infected with genotype 2 but all of them with a different and previously undescribed subtype[364], while this diversity has also seen in other Western and Central African countries. These observations reflect the huge genetic diversity in genotypes 1, 2 and 4, while also hinting at the long term presence in human populations of HCV in these geographical regions[354]. Indeed the currently suggested model by these genotype distributions is that HCV has been endemic in sub-Saharan Africa and South-East Asia for a considerable time, while the infections found in Western and other non-tropical countries represents a fairly recent emergence of new infections due to the spread of blood products and IDU[365;366].

Despite the genetic diversity of the 6 HCV genotypes, the main features of HCV structure, replication, transmission and ability to establish persistent infection are shared by all known variants[354]. The fact that all 6 genotypes are widespread throughout the human population is evidence in itself that each is equally successful in maintaining infections in humans. However, there is growing evidence that there may be genotypic differences in persistence and interactions with the immune system that have repercussions for current and probable future treatment. The clearest indication of this difference can be seen in the susceptibility

Figure 2.2.5 Genotypic diversity of HCV[354]

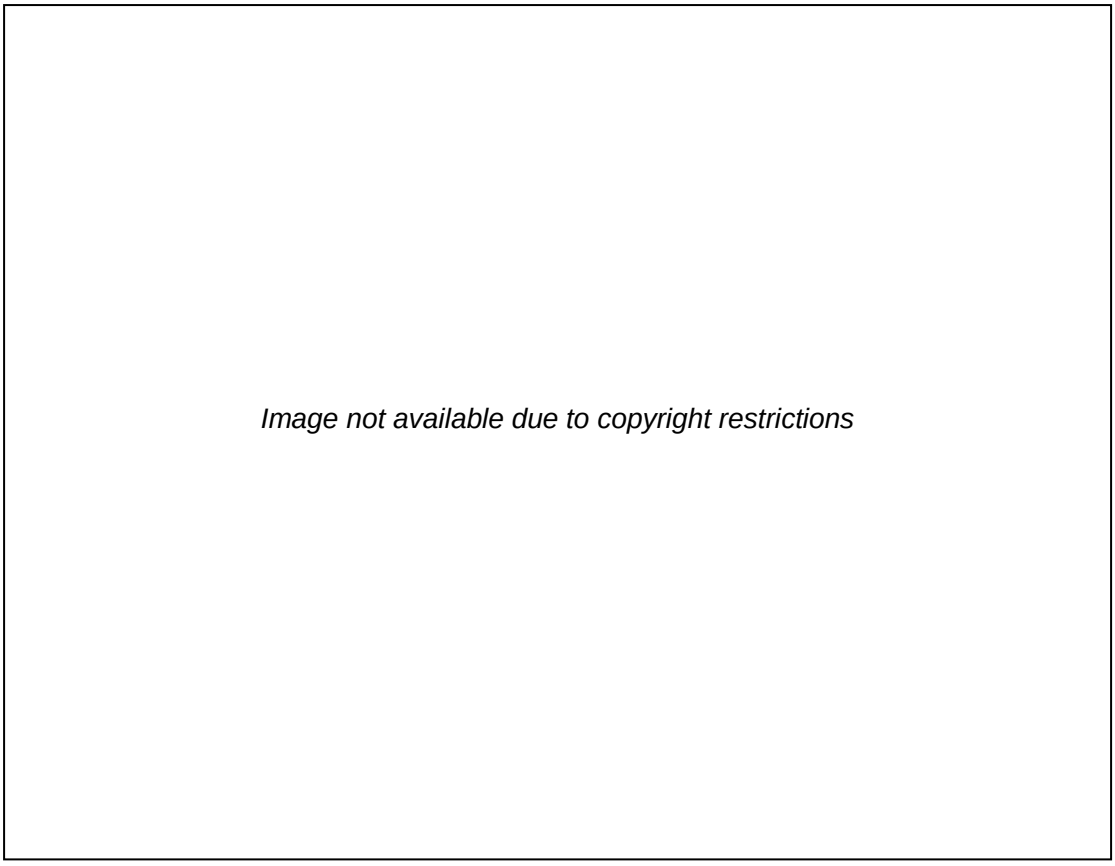
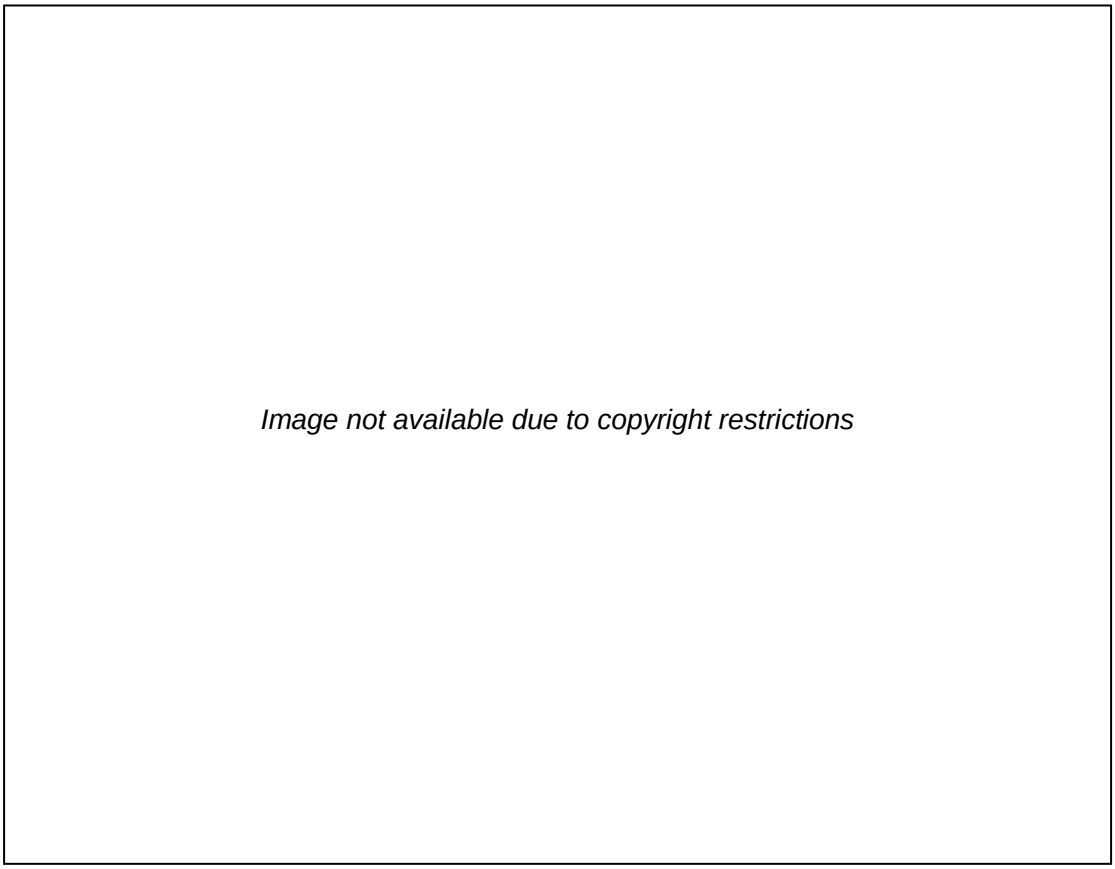


Figure 2.2.6 Genetic diversity of HCV subtypes in Africa and South-East Asia[354]



of the genotypes to treatment for HCV with pegylated-interferon in combination with ribavirin. For those with genotype 1, typically only 40%-50% of individuals on treatment will achieve complete and permanent clearance of the virus, whereas for genotypes 2 and 3 between 70% and 80% are expected to permanently clear the virus[367;368].

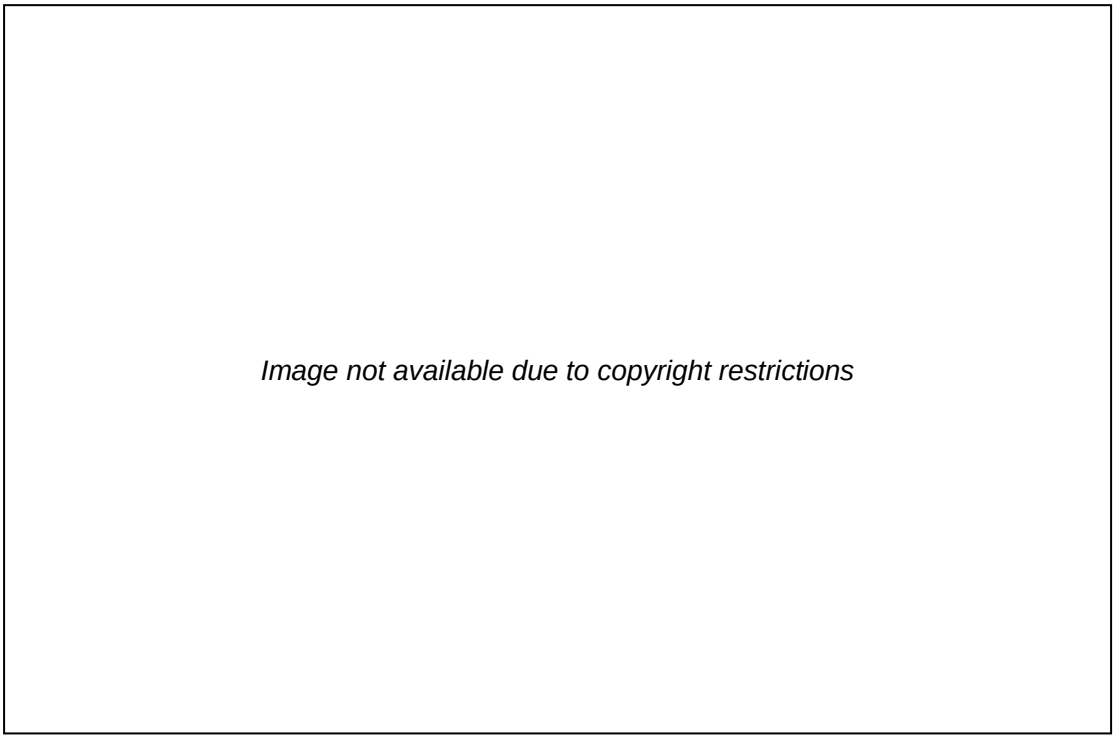
A further interesting feature of the rapid mutation rate of HCV within an infected individual is its ability to adapt rapidly to environmental changes. The large amount of genetic heterogeneity present in the viral pool of an infected individual has led to HCV being defined as existing as a Darwinian quasispecies within a host, meaning that it could have the ability to rapidly evolve. It's thought that this could be the reason why, through selection of virus with specific immune responses, HCV has seemingly found a niche in nature living human hosts. As a consequence of this it's thought that HCV infections will, in a similar fashion to HIV, be quick to establish resistance to ARV drugs that target specific areas of the HCV life cycle.

2.2.5 Clinical manifestation of HCV

Infection with HCV can cause both acute and chronic hepatitis[269;369]. In both cases HCV RNA can be detected in almost all patients within the first 2 weeks of infection, with HCV RNA levels rising rapidly during the first few weeks and then more slowly before reaching levels between 10^5 to 10^7 International Units (IU)/ml[369]. Serum alanine aminotransferase (ALT) levels, which are indicative of injury to the liver, start to rise 2 to 8 weeks after exposure and reach levels of 10 times the upper limit of the normal range[369;370]. In cases of self-limiting acute infection, symptoms may last for several weeks and begin to subside as ALT and HCV RNA levels being to fall[369]. Chronic hepatitis is defined by the persistence of HCV RNA for at least 6 months after the onset of infection[369]. It's estimated that between 75% and 85% of people infected with HCV will develop chronic infection and that 20% of those will develop liver cirrhosis[371].

HCV infection has been steadily assuming greater importance among HIV coinfected individuals. With the introduction of ART the rate of AIDS and death directly attributable to HIV has been declining (Section 2.1.6). Recent trend studies have shown increasing relative importance of HCV-related death over the years of 1995 and 2010 among the HIV population[372;373], as mortality rates for HIV-related death have declined rapidly over the same period. Other studies, mainly of HCV monoinfection, have also observed that for the first time it appears that the disease specific mortality rate associated with HCV surpassed that of HIV in 2007 (Figure 2.2.7)[374].

Figure 2.2.7 Annual age-adjusted disease specific mortality rates in the USA 1999-2007[374]



2.2.5.1 The effect of HIV on HCV

HIV adversely affects each stage of the natural history of HCV[375]. Following acute HCV infection, 85-95% of those with HIV will develop chronic disease, more in those with low CD4 counts[376-379]. This is significantly higher than the risk of chronicity among HCV monoinfected individuals, in whom approximately 30% will spontaneously clear the virus[380;381]. Similarly, HIV infection has been associated with higher HCV RNA levels and a more rapid progression of HCV-related liver disease[382-384]. *Eyster et al* reported that HCV RNA levels were higher in haemophiliacs who became HIV infected compared to those who remained HIV negative, while liver failure occurred exclusively in those with HIV/HCV coinfection[*383;384*]. Further, other studies of HIV/HCV coinfected haemophiliacs have largely found similar results[385-387]. A detailed description of the natural history of HCV RNA in HIV/HCV coinfected individuals and its relationship to clinical outcomes is provided in the introduction to Chapter 4.

In a case-control study of people mainly infected via injecting drug use it was found that HIV/HCV coinfected people had a greater extent of liver fibrosis than HCV monoinfected people after matching on factors that affect fibrosis progression, such as alcohol consumption[388]. A large meta-analysis of eight separate studies that investigated the role of HIV on liver disease in HCV coinfected people also found that coinfected individuals had approximately twice the risk of cirrhosis and six times the risk of decompensated liver disease[389].

Similarly, a prospective study following HCV infected haemophiliacs estimated the 16 year cumulative incidence of end-stage liver disease (ESLD) among men with and without HIV to be 14% and 2.6%, respectively[390]. More recently the effect of HIV on HCV has been summarised in a meta-analysis which demonstrated that HIV coinfection was associated with 6-fold increased risk of ESLD and 2-fold increased risk of histological cirrhosis compared to HCV monoinfection[391]. Further, a cohort study looking at the short term prognosis of HIV/HCV coinfected individuals found that those with cirrhosis have a relatively good 3-year survival probability of 87%, whereas the 2-year survival of those with decompensated liver cirrhosis was only 50%[392].

Although it is well-established that low CD4 cell counts are associated with faster progression of liver fibrosis[393;394], the mechanism by which accelerated fibrosis progression occurs in HIV/HCV coinfected people is not well understood. However, there are several hypotheses, including a direct viral effect of HIV on the hepatocytes and many immunologic alterations such as diminished HCV specific T-cell responses[376;395]. Evidence of this hypothesis is given by studies which have shown that effective treatment for HIV among coinfected individuals can also have an impact on the rate of mortality associated with HCV. In 2003 a study of 285 coinfected individuals with follow-up over the period 1990-2002, spanning the introduction of effective treatment for HIV, showed that treatment with monotherapy, or in particular cART was not only associated with reduced overall mortality but also reduced liver-related mortality (Figure 2.2.8)[396].

However, successful treatment for HIV does not appear to completely reverse the impact of coinfection. A large recent study with follow-up between 1997-2010, including over 4,000 coinfected individuals taking HIV therapy and over 6,000 HCV monoinfected individuals, has shown that coinfected individuals that maintain low HIV viral loads due to effective treatment still remain at increased risk of hepatic decompensation compared to HCV monoinfected individuals[397]. The authors estimate that the incidence of hepatic decompensation is 1.5 times higher among coinfected individuals with controlled HIV infection compared with HCV monoinfected individuals (Figure 2.2.9)[397].

2.2.5.2 The effect of HCV on HIV

There are conflicting reports on the effect of HCV infection on the natural history of HIV disease[380]. Before the HAART era, in a prospective study of 416 HIV seroconverters, HCV coinfected persons had similar HIV progression rates to those without HCV infection[398]. While in another study of 1,955 individuals it was found that HCV infection was not independently associated with progression to AIDS of death, after adjusting for exposure to HAART and HIV RNA suppression[399]. Similarly, among 823 patients followed in the HIV

Figure 2.2.8 The effect of HIV treatment on all cause and liver-related mortality among coinfected individuals[396]

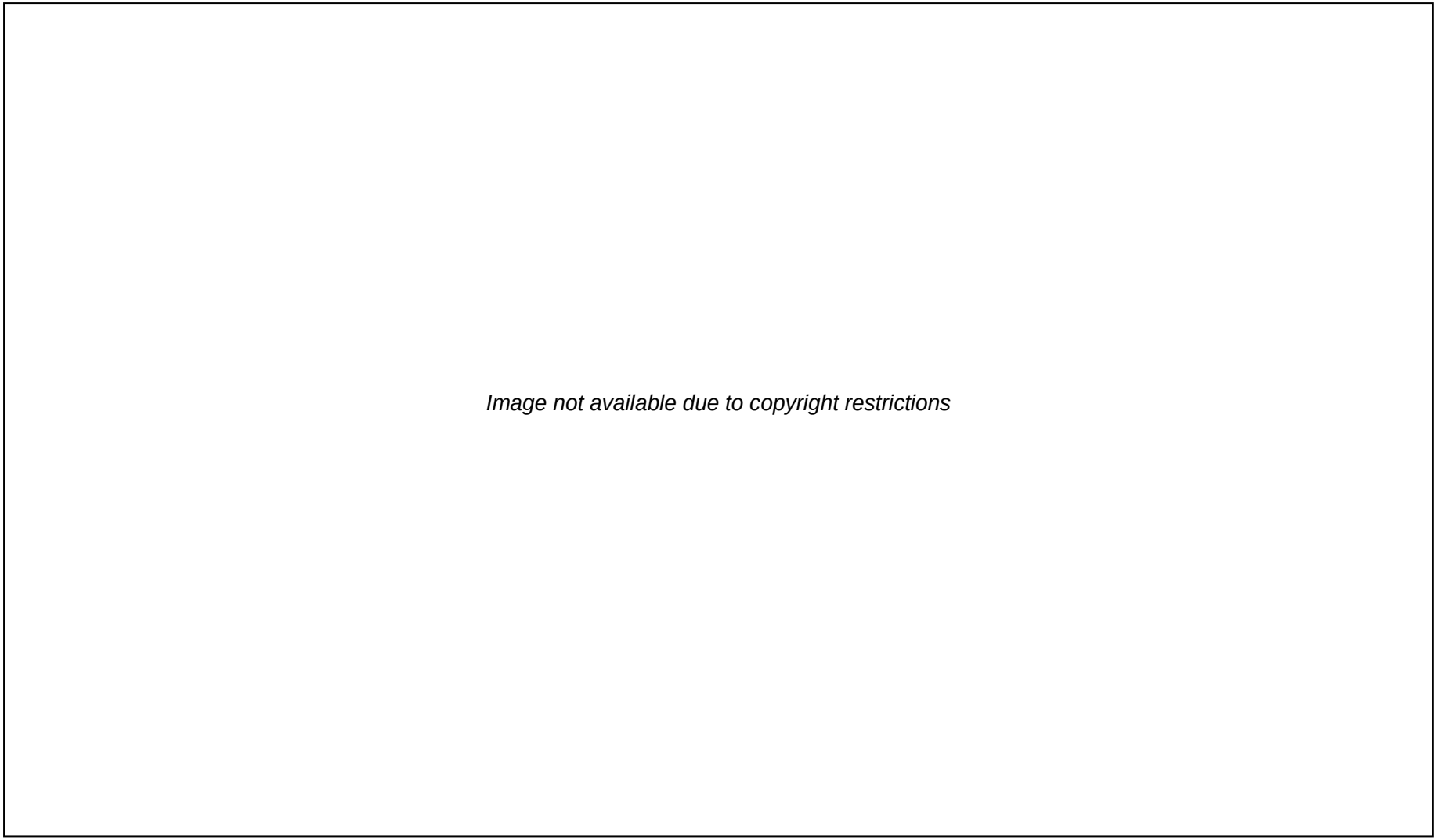
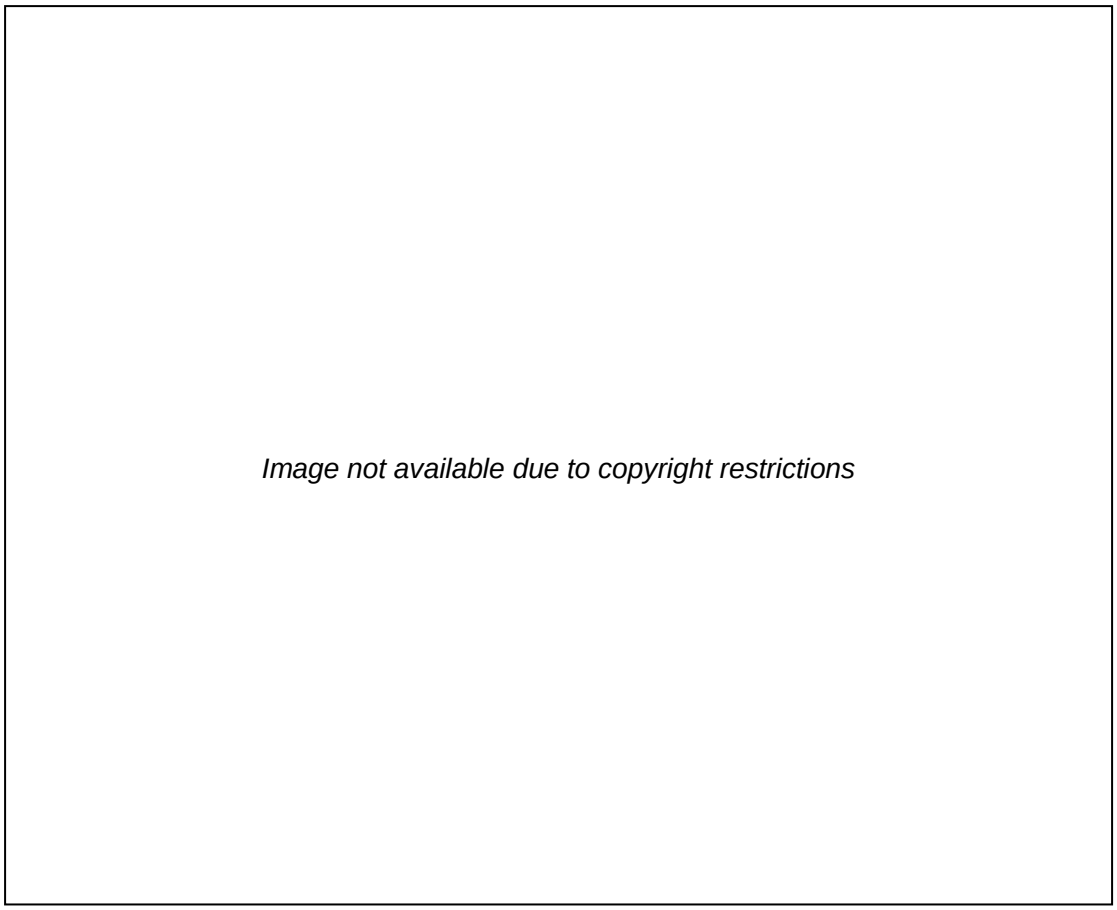


Figure 2.2.9 Cumulative incidence of hepatic decompensation for HCV monoinfected and ARV-treated coinfected individuals[397]



Outpatient Study it was found that HCV coinfection was not associated with survival[400]. On the other hand, a large study of 3,111 patients receiving HAART reported that HCV coinfected individuals had a modestly increased risk of progression to a new AIDS defining event or death, even among the subgroup with continuous suppression of HIV RNA[401].

Multiple studies have also looked at the effect of HCV coinfection on CD4 rebound after initiation of HAART. The Swiss HIV cohort study found that in the first year of HAART, those coinfected with HCV had smaller increases in CD4 lymphocytes than HCV seronegative individuals, but this difference disappeared during the 4 year follow-up of the study[402]. The EuroSIDA study on the other hand did not find an effect of HCV coinfection on HIV disease progression or CD4 cell recovery[403;404]. Further, a review of eight cohort studies showed that the CD4 cell count response for patients with HIV/HCV coinfection after they started receiving HAART was 33.4 cells/ml lower than those monoinfected with HIV, on average[405]. In addition, among a cohort of coinfected women it was shown that HIV/HCV coinfection was associated with an increase in a subset of CD4 and CD8 memory cells but did not alter the total number of CD4 cells or the immune response to HAART[406].

The conflicting data on the relationship between HCV coinfection and HIV disease outcome is thought to be the result of confounding factors, mainly injecting drug use in patients with HCV, which may limit the effectiveness of HAART[380]. The mechanisms by which the two viruses interact at cellular level remain largely unexplored[376]; however, despite this and the conflicting cohort study results, at present the overall literature suggests that the major contribution of HCV to mortality in coinfected individuals is attributable to accelerated liver disease and not an increased incidence of AIDS-related complications[403;407].

Mortality associated with chronic HCV infection results mainly from the development of liver fibrosis and the subsequent occurrence of cirrhosis and ESLD, while further progression to hepatocellular carcinoma (HCC) is associated with a very high rate of mortality[408]. HCC is the third leading cause of cancer mortality worldwide and although 2- to 4-year survival rates have doubled in the general population since the early 1990s, due to screening and earlier diagnosis, the 1-year survival rate remains less than 50%[409]. Consequently, documentation of the level and rate of progression of liver fibrosis is essential in the management of persons infected with HCV.

The rate and speed of disease progression from chronic infection to cirrhosis and on to HCC varies depending on many factors[369;378]. Though studies on the natural history of HCV infection in immunocompetent people have demonstrated cirrhosis typically develops 20-30 years after the first exposure to the virus[408;410;411], females and those who are young at the time of infection typically experience slow progression, more than 30 years to cirrhosis, while alcohol users and those coinfected with HIV typically experience faster progression, less than 20 years to cirrhosis[371]. Approximately 6% of monoinfected HCV individuals can be expected to develop hepatic decompensation due to cirrhosis during a 20 year period[412], while for HIV/HCV coinfected individuals it is estimated that the risk of cirrhosis or hepatic decompensation is around 3 times greater[413]. Once cirrhosis has been established it is estimated that the risk of HCC developing is between 1% and 4% per year[371].

Progression of liver fibrosis

The rate of fibrosis progression is dependent upon a number of factors and is often specific to each individual. Among HCV monoinfected individuals progression of liver fibrosis is known to be affected by age, alcohol consumption and HCV genotype. Progression rates from F0 to cirrhosis for HCV monoinfected individuals are shown in Table 2.2.1.

In HIV/HCV coinfected individuals a number of HIV-related factors are known to further influence the rate of liver fibrosis progression. As mentioned above, low CD4 cell counts have been consistently associated with faster progression of liver fibrosis[393;394]. One study

Table 2.2.1 Rates of liver fibrosis progression[408]

Risk factors		Number of patients	Rate of fibrosis progression per year	Expected duration for progression to cirrhosis (years)
One factor classification				
Age at infection				
	≤20	268	0·091 (0·083 to 0·100)	44 (40–48)
	21–30	404	0·105 (0·100 to 0·125)	38 (32–40)
	31–40	183	0·138 (0·111 to 0·160)	30 (25–36)
	41–50	166	0·200 (0·174 to 0·231)	20 (17–23)
	>50	136	0·333 (0·272 to 0·375)	12 (11–15)
Daily alcohol consumption (g)				
	0	598	0·125 (0·111 to 0·143)	32 (28–36)
	1–49	330	0·143 (0·118 to 0·160)	28 (25–34)
	≥50	111	0·167 (0·133 to 0·174)	24 (23–30)
Sex				
	Female	517	0·111 (0·100 to 0·125)	36 (32–40)
	Male	639	0·154 (0·143 to 0·167)	26 (24–28)
Genotype				
	1a	44	0·128 (0·091 to 0·200)	31 (20–44)
	1b	111	0·091 (0·080 to 0·111)	44 (36–50)
	2	30	0·088 (0·059 to 1·125)	45 (32–68)
	3	39	0·167 (0·125 to 0·222)	24 (18–32)
	4–5–6	22	0·167 (0·098 to 0·250)	24 (16–41)
Three factors classification				
Age at infection ≤40 years				
	Alcohol <50 g			
	Female	313	0·095 (0·088 to 0·100)	42 (40–45)
	Male	362	0·111 (0·091 to 0·130)	36 (31-44)
	Alcohol ≥50 g			
	Female	13	0·083 (0·043 to 0·111)	N/A
	Male	77	0·154 (0·125 to 0·167)	26 (24–32)
Age at infection >40 years				
	Alcohol <50 g			
	Female	136	0·200 (0·167 to 0·250)	20 (16–24)
	Male	116	0·301 (0·235 to 0·333)	13 (12–17)
	Alcohol ≥50 g	21	0·267 (0·200 to 0·500)	15 (8–20)
	Female	4	0·633 (−0.489 to 2·206)	N/A
	Male	17	0·250 (0·109 to 1·117)	N/A
All patients with duration of infection		1157	0·133 (0·125 to 0·143)	30 (28–32)

N/A=not available because sample size was too small. All values are median (95% CI).

Except for genotypes, there was a significant difference between medians for all classifications (*P*<0·05).

by *Benhamou et al* showed that CD4 cell counts <200cells/mm^3 were associated with a 6.5-fold increased risk of liver fibrosis progression[393]. Studies have also shown that HIV/HCV coinfected individuals with HIV viral loads above 400copies/ml have faster fibrosis progression rates than HCV monoinfected individuals[414]. However, the same study found that there was no difference between fibrosis progression rates of HCV monoinfected and HIV/HCV coinfected individuals when HIV viral load was undetectable[414]. Consequently, treatment with cART, which lowers HIV viral load and increases CD4 cell count, has been found to be protective against rapid progression of liver fibrosis in coinfected individuals[415]. As a result, current European treatment guidelines recommend early initiation of cART for HIV/HCV coinfected individuals to avoid time spent with low CD4 cell counts[416].

2.2.5.3 Estimation of liver fibrosis

A number of classification systems exist for quantifying degrees of liver fibrosis, including the ISHTAK and Batts-Ludwig classification systems[417;418]. However, recently the METAVIR scoring system has become the prominent classification system in HIV/HCV coinfected individual research. In the mid-nineties the METAVIR group of 10 senior French pathologists experienced in the field of liver pathology developed a standard set of criteria for the grading and classification of fibrosis stage[419;420]. The assessment classifies liver fibrosis into five stages of increasing severity (F0, F1, F2, F3 and F4)[419;420]. The specific definitions of the stages are as follows F0 no fibrosis, F1 stellate enlargement of portal tract but without septa formation, F2 enlargement of portal tract with rare septa formation, F3 numerous septa without cirrhosis, F4 cirrhosis[419;420]. More recently, any level of fibrosis graded as ≥F2 has been defined as significant fibrosis[421]. The scoring system has been validated and studies of HCV monoinfection have determined that the risk of progression from one stage to the next in each year is in the region of 13% (95% CI 12.5 – 14.3)[408;419;420].

Fibroscan

Traditionally, histological examination of liver biopsy was considered to be the gold standard for evaluating hepatic fibrosis[421;422]. However, liver biopsy is an invasive and painful procedure, often with poor patient acceptance and also carries a small risk of complications and potentially life-threatening consequences[421;423]. The accuracy of liver biopsy has also been called into question as there is potential for observer bias and studies have shown that even experienced physicians have a 20% error rate in disease staging[424].

Fortunately, a non-invasive method of liver fibrosis evaluation was developed in 2008 using transient elastography (TE) to measure liver stiffness[421]. TE is a rapid and user-friendly technique that can be performed at the bedside or outpatient clinic with immediate results

and good reproducibility[421]. Marketed as Fibroscan® (Echosens, Paris, France), TE is now commonly used to determine liver fibrosis levels with scores below 7.0 kiloPascals (kPa) equivalent to absent or mild fibrosis (METAVIR levels F0-F1), scores between 7.0 - 9.5kPa reflect significant fibrosis (F2), scores between 9.5 – 12.5 indicate severe fibrosis (F3) while scores above 12.5kPa are attributed to cirrhosis (F4)[421].

AST to platelet ratio index (APRI)

Prior to the development of Fibroscan®, other non-invasive measures of liver fibrosis used widely available laboratory measurements which were known to be affected by the presence of liver fibrosis. One such measure is the AST to platelet ratio index (APRI) which was developed in 2003[425]. The study by *Wai et al* initially described AST and platelets to be the most important predictors of fibrosis from a list of standard laboratory measurements[425]. The authors noticed that there was significant overlap between AST and platelets for individuals with different stages of liver fibrosis. Therefore, the authors attempted to amplify the difference between AST and platelets with a new novel index given below, where ULN is the upper limit of the normal range for AST[425].

$$APRI = \frac{AST\ level\ (/ULN)}{Platelets\ (10^9/L)} \times 100$$

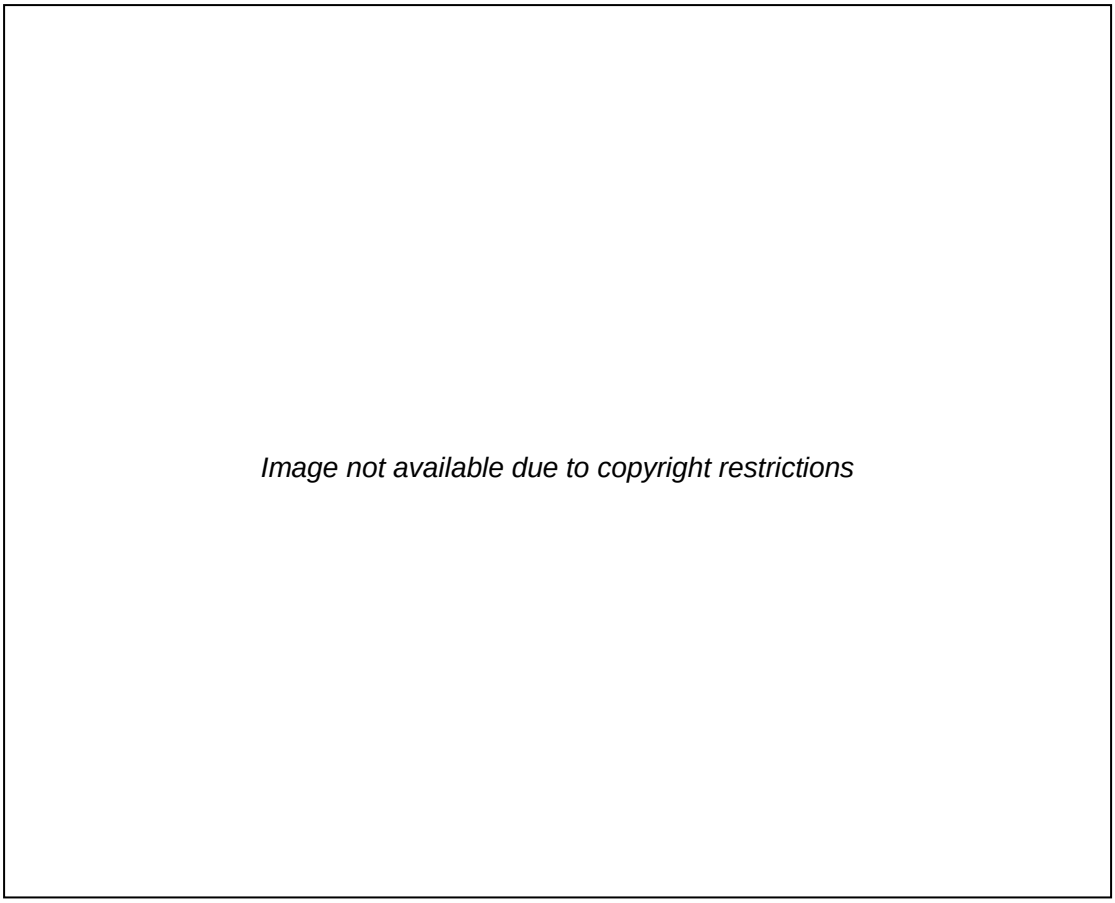
The authors discovered that this new index performed significantly better than AST or platelets alone at determining significant liver fibrosis, with values above 1.5 providing the optimum level of prediction[425]. Following publication of this novel index, the APRI score was validated in a number of studies which have shown its accuracy as a non-invasive marker of fibrosis and it continues to be used today where other measures are not available[426].

2.2.6 Treatment

2.2.6.1 The history of treatment for HCV

Treatment for HCV has traditionally revolved around the use of interferon (IFN). IFNs are natural cellular proteins that perform a variety of functions, including the induction of an antiviral state in their target cells, recruitment of immune cells, and induction of cell differentiation[367]. Since the first clinical trials of IFN-alpha treatment in chronic HCV patients in the mid-1980s[427], dose regimens have been progressively refined (Figure 2.2.10)[367]. Monotherapy with three megaunits of IFN three times a week for 24 weeks was associated with a 90% failure rate, which was reduced to 84% when treatment was extended to 48 weeks[428]. The addition of ribavirin to treatment regimens reduced the failure rate to

Figure 2.2.10 Dosage refinement and treatment failure of IFN therapy[367]



approximately 60%, while switching to pegylated-IFN once a week from standard IFN-alpha reduced failure rates by another 10%[428-430].

Pegylated-IFN is an IFN molecule linked to a polyethylene glycol molecule to ensure sustained IFN concentrations after weekly injections[367]. Ribavirin is a synthetic guanosine analog that has only a moderate effect on HCV *in vivo*[431], but has a boosting effect on IFN by modulating the immune response and accelerating the clearance of infected cells, which is taken orally daily[367]. Treatment regimens were further optimised by adjusting doses of ribavirin and, in certain cases, pegylated-IFN to the body weight of recipients[430;432;433]. The success of pegylated-IFN plus ribavirin treatment reached a minimum failure rate of approximately 30% (40% for HCV genotype 1 and 10% for genotypes 2 or 3) when full adherence was ensured in clinical trials[432;433]. However, such low failure rates were never replicated in routine practice while this combination remained the gold standard for HCV treatment up to 2010[367;434-436].

2.2.6.2 Current HCV treatment regimens and guidelines

In 2011 the first direct-acting antivirals (DAA) for HCV, boceprevir and telaprevir, became available for treatment of HCV genotype 1 infection[437-439]. In clinical trials these drugs have

seen cure rates for HCV genotype 1 rise to 75% in treatment naïve patients[437;440] and 60% in treatment experienced patients[437;441;442]. However, treatment with these early DAAs still retains the backbone of pegylated-IFN and ribavirin, referred to as triple therapy[437;442].

IFN is not well tolerated and causes flu like symptoms, while being contraindicated with some HIV medications[443]. Consequently, the uptake of treatment with boceprevir and telaprevir for HCV remained low with few patients eligible for therapy, typically less than 30%[443;444]. Further, the estimated costs of triple therapy for HCV genotype 1 of approximately €30,000 proved restrictive in countries in the low income setting[2]. However, drug development for the treatment of HCV continues to be a rapidly advancing field, with many 2nd generation agents undergoing phase II and phase III clinical trials[445]. These new agents are expected to herald a new era of treatment for HCV with cure rates far in excess of those seen previously, approaching 90-100%[445;445].

The year 2014 saw the approval of a number of 2nd generation DAAs for treatment of HCV[446]. These new drugs have shown superior treatment efficacy and fewer side effects in

Table 2.2.2 EMA licensed IFN-free HCV treatment recommendations[416]

HCV Genotype	***DAAs***	***Treatment duration***	***EMA approval date[252]***
1 & 4	Sofosbuvir + Ribavirin Sofosbuvir + Simeprevir Sofosbuvir + Daclatasvir	24 weeks[1] 12 weeks[2] 12 weeks: non-cirrhotics 24 weeks: with cirrhosis	Sofosbuvir: Jan 2014 Simeprevir: May 2014 Daclatasvir: Aug 2014
2	Sofosbuvir + Ribavirin	12 weeks[3]	
3	Sofosbuvir + Ribavirin Sofosbuvir + Daclatasvir + Ribavirin	24 weeks 24 weeks for cirrhotics and treatment experienced	
5 & 6	Without clinical trials data genotypes 5&6 are suggested to be treated similarly to genotypes 1&4		

[1]Only licensed for those ineligible for IFN-containing treatment

[2]Can be extended to 24 weeks for treatment experienced cirrhotics, with or without ribavirin

[3]Can be extended to 16 weeks for treatment naïve cirrhotics; 24 weeks for treatment experienced

most cases, and IFN-free combinations of DAAs are now seen as the gold standard of therapy in HCV[416]. Further, treatment durations for these new drugs are potentially far reduced, often between 12 and 24 weeks for some drug combinations and HCV genotypes[416]. However, the second generation DAAs are currently even more expensive than the first, with treatment costs approaching €100,000 for all oral IFN-free DAA combinations. Consequently, the use of DAA therapy as the gold standard of care is currently limited by each country and clinical centre's ability to pay for them and IFN-containing regimens are still used in some circumstances[416].

Current European treatment guidelines from the European AIDS Clinical Society (EACS) recommend that IFN-free DAA combinations should be considered the standard of care for HCV treatment, especially in those who have advanced levels of liver fibrosis[416]. Specific treatment regimens and treatment durations vary depending on HCV genotype, the level of liver fibrosis and previous treatment history. Table 2.2.2 outlines current IFN-free HCV treatment recommendations. In brief, for HCV genotype 2 a combination of sofosbuvir plus weight-based ribavirin for 12 weeks is recommended, while for genotype 3 this regimen may be extended to 24 weeks[416]. For genotypes 1 and 4 a combination of sofosbuvir plus simeprevir is recommended for 12 weeks and should be extended to 24 weeks for treatment experienced cirrhotic individuals, with or without ribavirin[416]. The combination of

Table 2.2.3 EMA licensed IFN-containing HCV treatment recomendations[416]

HCV Genotype	***DAAs***	***Treatment duration***	***EMA approval date[252]***
1 & 4	Sofosbuvir + PEG-IFN/RBV Simeprevir + PEG-IFN/RBV Daclatasvir + PEG-IFN/RBV	12 weeks[1] 24 weeks[2] 24 weeks/48 weeks[3]	Sofosbuvir: Jan 2014 Simeprevir: May 2014 Daclatasvir: Aug 2014
2	PEG-IFN/RBV	24 weeks/48 weeks[3]	
3	Sofosbuvir + PEG-IFN/RBV	12 weeks[1]	
5 & 6	Without clinical trials data genotypes 5&6 are suggested to be treated similarly to genotypes 1&4		

[1]Can be extended to 24 weeks for cirrhotics

[2]Extended to 48 weeks for cirrhotics, treatment experienced and relapsers

[3]24 weeks if there is a rapid response, otherwise 48 weeks

sofosbuvir and daclatasvir is recommended for all genotypes for 12 weeks in the absence of cirrhosis, with an extension to 24 weeks with cirrhosis[416].

Where there is limited availability or affordability of DAAs and combination IFN-free treatment is not possible, sofosbuvir plus pegylated-interferon and ribavirin for 12 weeks is considered the best alternative for genotypes 1, 3-6, and treatment should be extended to 24 weeks for cirrhotics[416]. When sofosbuvir is not available then simeprevir plus pegylated-interferon and ribavirin for 24 weeks can be used for genotypes 1 and 4, while a dual course of pegylated-interferon and ribavirin may be considered for 24 weeks for genotype 2[416]. Table 2.2.3 outlines current IFN-containing HCV treatment recommendations.

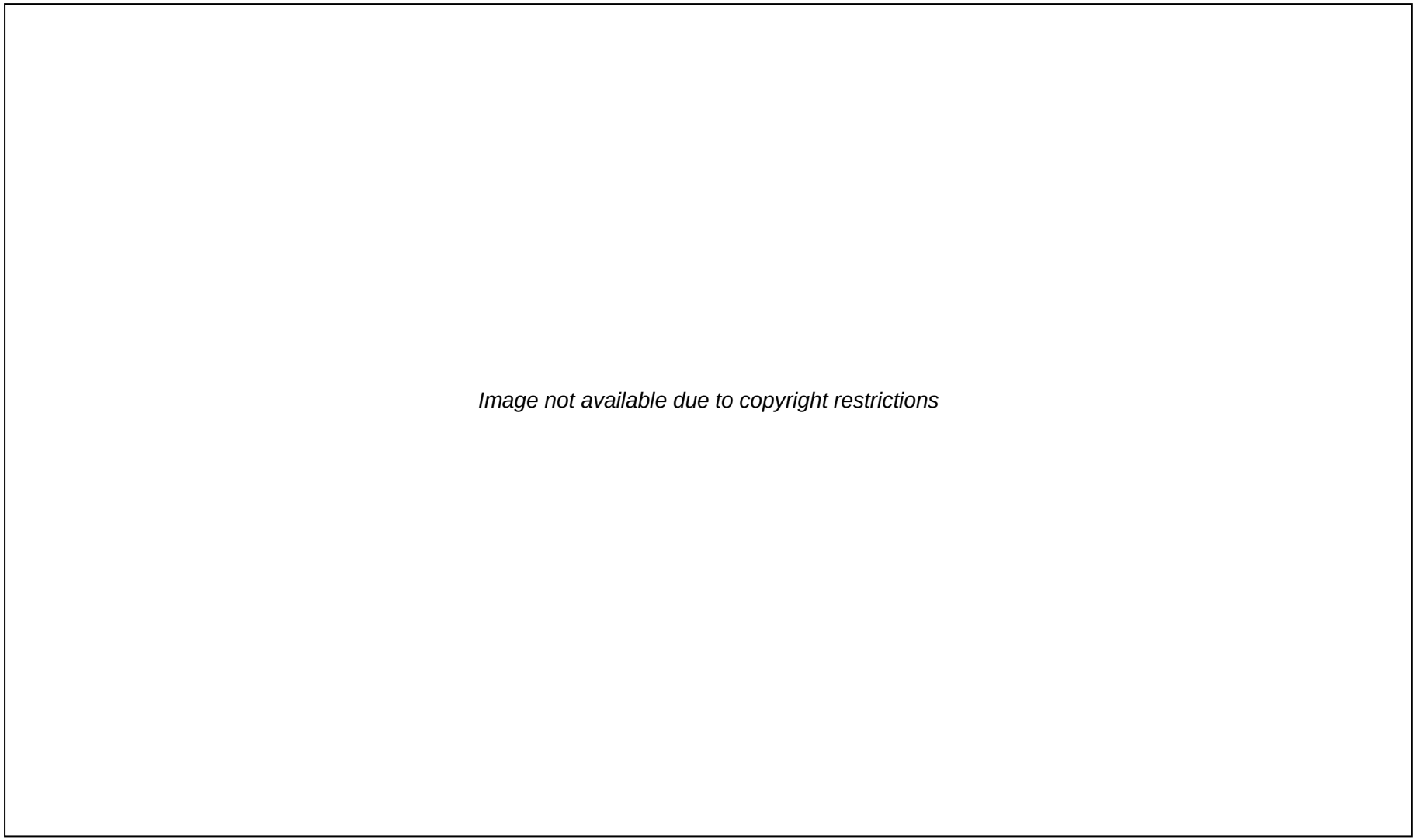
Finally, when second generation DAAs are not available and will not be available for some time, the first generation DAAs boceprevir and telaprevir may be used for genotype 1 following response guided therapy[416]. Lead in times and stopping rules for DAA combinations with pegylated-interferon and ribavirin are shown in Figure 2.2.11.

2.2.6.3 HCV treatment stages and response-guided therapy

HCV does not integrate with the host genome of an infected cell (Section 2.2.3). Therefore, successful treatment for HCV which is defined as sustained virologic response (SVR), is akin to cure[367;447]. HCV RNA viral load measurements taken during and after treatment are then used to categorise patients into different viral response groups. SVR is defined as HCV RNA undetectability 24 weeks after the end of therapy[367;448]. Although not a perfect definition of a cure, it has been shown that 98% of people that achieve SVR are cured of HCV[449]. Patients that do not achieve SVR are said to be in 'treatment failure', which can correspond to different virological patterns.

Non-responders have no significant fall in HCV RNA load (>1 log) at any point during treatment. Partial responders have a significant drop in HCV RNA during treatment, but HCV RNA remains detectable. Responder-relapsers become HCV RNA negative during treatment but relapse after treatment withdrawal. Finally, responders with breakthrough initially become HCV RNA negative but relapse during treatment, although these definitions lack clear precision as other factors such as dose reduction or discontinuation and poor adherence can all affect viral response[367]. Classifications of more use in the clinical setting are the rapid virological responder (RVR), which is defined by undetectable HCV RNA 4 weeks after treatment initiation, the early virological response (EVR), defined by detectible HCV RNA at week 4 but undetectable at week 12, and the delayed virological responder (DVR), a greater than 2 log drop but detectible HCV RNA at week 12 and undetectable

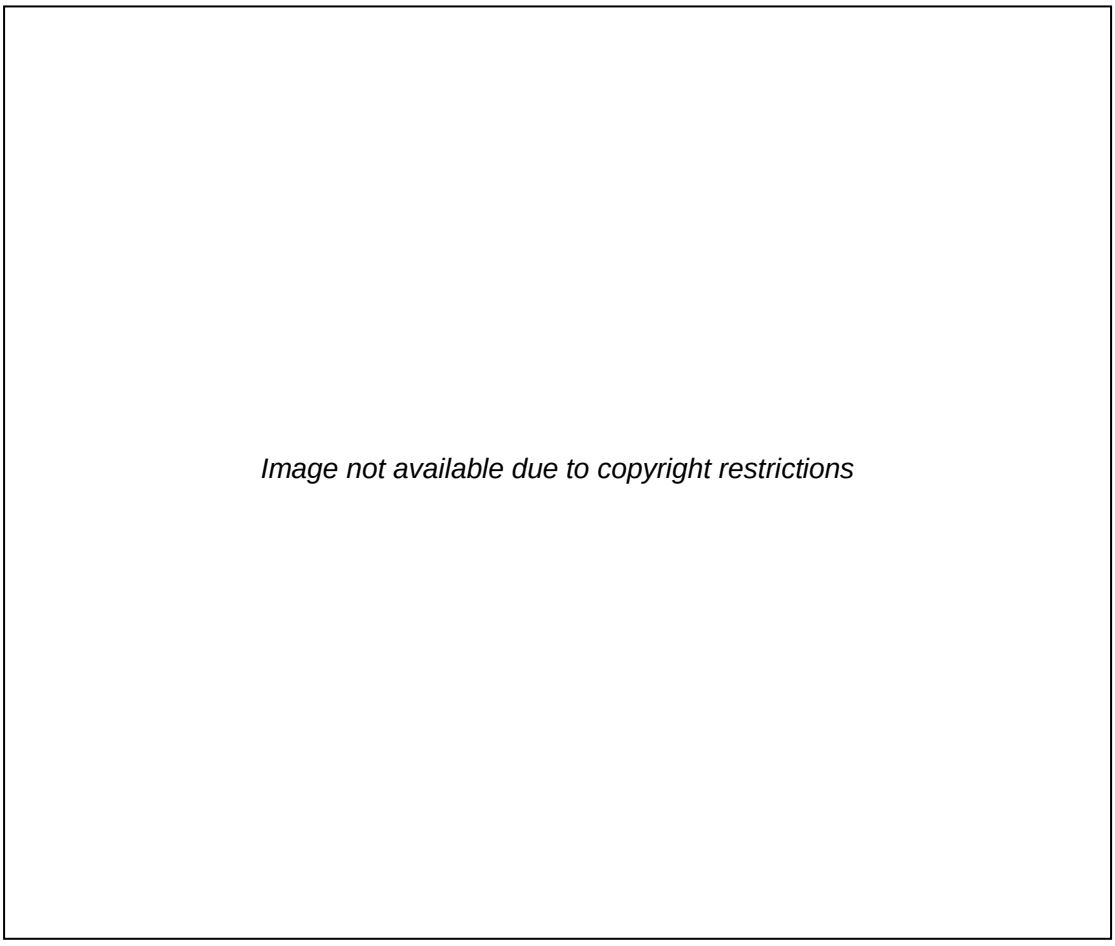
Figure 2.2.11 Recommended use of Telaprevir, Boceprevir, Simeprevir or Sofosbuvir with PEG-IFN and Ribavirin[416]



HCV RNA at week 24[450]. A graphical illustration of HCV treatment responses can be seen in Figure 2.2.12.

In practise treatment discontinuation decisions are made depending on whether a 2 log drop in HCV RNA has been observed by week 12 of treatment[367;450]. It has been shown that a fall of 2 log or greater (i.e. a 100-fold fall in baseline HCV RNA load) by week 12 of treatment has an excellent negative predictive value for SVR (98%-100%)[451], which implies that people who do not achieve a 2 log fall in HCV RNA by week 12 have virtually no chance of achieving SVR. Treatment is also often stopped in persons with detectible HCV RNA at week 24, as these people also have a minimal chance of SVR (1%-3%)[450;452;453]. HCV RNA measurements taken at baseline, week 4, week 12 and week 24 are then used to classify people as RVR, EVR or DVR and this information combined with other factors, such as the genotype of HCV infection, is used to determine whether to discontinue treatment, treat for 24 weeks, 48 weeks or in some cases 72 weeks. In general those with slower falls in HCV RNA, genotype 1 and contraindication to triple therapy with DAAs are treated for longer, as summarised in Figure 2.2.13[193].

Figure 2.2.12 HCV treatment virologic responses[454]



Probability of treatment success

The probability of achieving SVR depends on a number of factors, including HCV genotype, the IL28B polymorphism genotype, stage of fibrosis, HIV coinfection, baseline HCV RNA and host factors, with younger age, lower body mass index and female gender all associated with a better treatment response[367;450]. However, the potency of 2nd generation DAAs appears to have overcome most of these factors and they are of most relevance to treatment with pegylated-IFN and ribavirin alone[446].

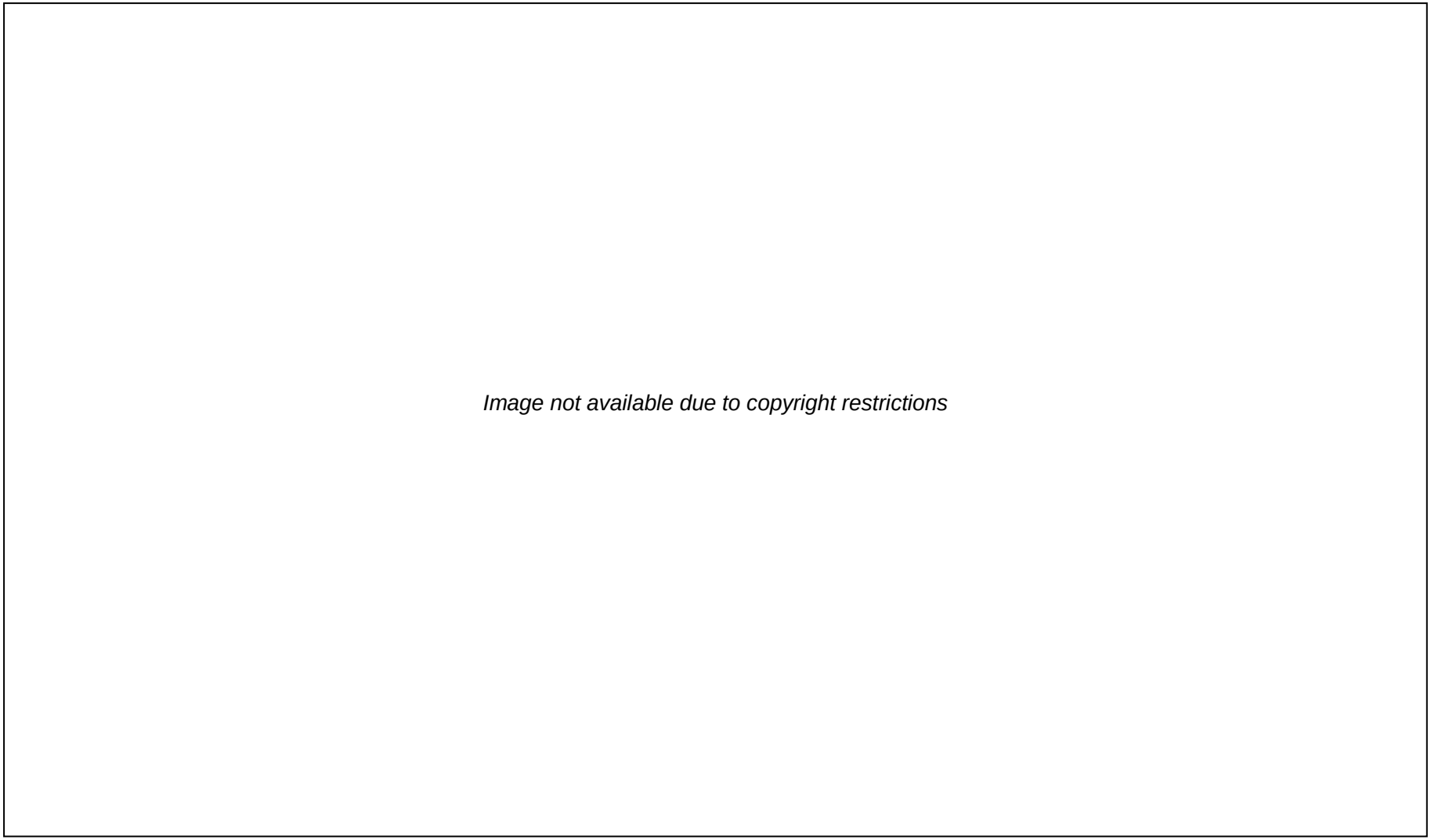
When treated with pegylated-IFN and ribavirin rates of SVR among HCV monoinfected individuals are typically between 40%-45% for HCV genotype 1 and slightly higher for genotype 4[451;455;456], when treated with triple therapy including either boceprevir or telaprevir rates of SVR increase to 75%[439]. For genotypes 2 and 3 SVR is more likely with rates of between 75%-80% reported[457;458]. However, among those coinfected with HIV, SVR falls to between 17%-32% for those with genotypes 1 and 4, and between 44%-73% for genotypes 2 and 3[434;435;448;459]. The IL28B polymorphism is in the vicinity of IFN genes on human chromosome 19 and has been linked with SVR in HCV. IL28B can take 3 different genotype forms, CC, CT, and TT. The CC genotype, which is more common in Caucasians, has been found to be associated with spontaneous clearance of HCV[460], while rates of SVR have also been found to be higher in those with the CC genotype, with 30% achieving SVR with non-CC genotypes and up to 70% in those with the CC genotype[461;462].

In comparison, modern DAA containing regimens have shown excellent HCV cure rates regardless of IL28B genotype and individual level factors. Two studies evaluating sofosbuvir taken with pegylated-IFN and ribavirin for 12 weeks found SVR rates between 89% and 91% for HCV genotype 1[463;464]. While a recent study including difficult to treat patients, previous non-responders and those with advanced liver fibrosis, found that the combination of sofosbuvir and simeprevir with or without ribavirin for 12 or 24 weeks had an overall SVR rate of 92% for HCV genotype 1[465].

2.2.7 Reinfection

HCV is different from other viral infections in that infection and the generation of an immune response does not necessarily protect an individual from reinfection[466]. There has been some disagreement between studies looking at the risk of reinfection with HCV, with rates of clearance following reinfection ranging from 29%[467], similar to the rate of clearance of primary infection, to 90%[468]. However, these differences can probably be explained by study design and most would now agree that clearance of HCV offers some level of protection against reinfection[469].

Figure 2.2.13 Optimal response-guided therapy for those not eligible for triple therapy with DAAs[193]



Studies of IDUs, who are at high risk of reinfection, have produced mixed results. Some studies have shown that reinfection with HCV after viral clearance is more common in those to have been previously infected compared to HCV-naïve patients[466;470]. Other studies have shown that reinfection rates are lower among those previously infected and suggest that there is some residual protection against reinfection following viral clearance[467;471]. However, the rate of reinfection after clearance of HCV is high in all studies of IDUs, with rates of reinfection approaching 50%[466;472]. Studies assessing the risk of HCV reinfection among MSM are less common, however, they also suggest that reinfection is frequent in this risk group, reporting cumulative risk of reinfection of 33% in the first two years following the initial infection and clearance[473;474].

Given the high rate of chronicity of HCV infection and the sizeable expense of treating the infection, reinfection rates for HCV are alarming[472]. Further, the lack of data suggesting immunity following initial infection complicates the development of an effective HCV vaccine[471]. Therapeutic HCV vaccine efficacy has so far been very poor, however, research continues in this important area with many vaccine candidates, which aim to induce a cellular immune response, under development in preclinical study[471;475;476].

2.2.8 Hepatitis B Virus

Hepatitis B virus (HBV) is a member of the hepadnavirus family, simply meaning the hepatic DNA virus family[477;478]. HBV is one of the smallest animal viruses with each viron no more than 42 nanometres (nm) in diameter[477;478]. The virus consists of an outer lipid envelope containing the surface antigen and a nucleocapsid core, made of protein, containing a partially double stranded viral DNA and DNA polymerase, with reverse transcriptase activity (Figure 2.2.14)[477;478]. The virus releases three glycoproteins, S, L and M. The S protein is the most frequently produced[479]. The L and M proteins are based on the S structure and are produced in only 5-15% and 1-2% of the frequency of the S protein, respectively[479]. In the replication process, along with fully infectious particles, other non-infectious particles made from the lipid layer and protein but without a core, known as the hepatitis B surface antigen (HBsAg), are produced in excess and released into the blood stream[477-479]. Consequently, tests for HBsAg are now commonly used to determine whether a person is infected with actively replicating HBV.

2.2.8.1 Epidemiology

HBV is one of the world's most common infectious diseases with a global distribution[480;481]. The HBsAg carrier rate ranges from 0.1% to 20% in different populations around the world (Figure 2.2.15)[482]. It is estimated that one third of the world's population has been infected with HBV[480;481]. Approximately 5% are chronic carriers and approaching 25% of all chronic

Figure 2.2.14 Structure of the hepatitis B virus[271]

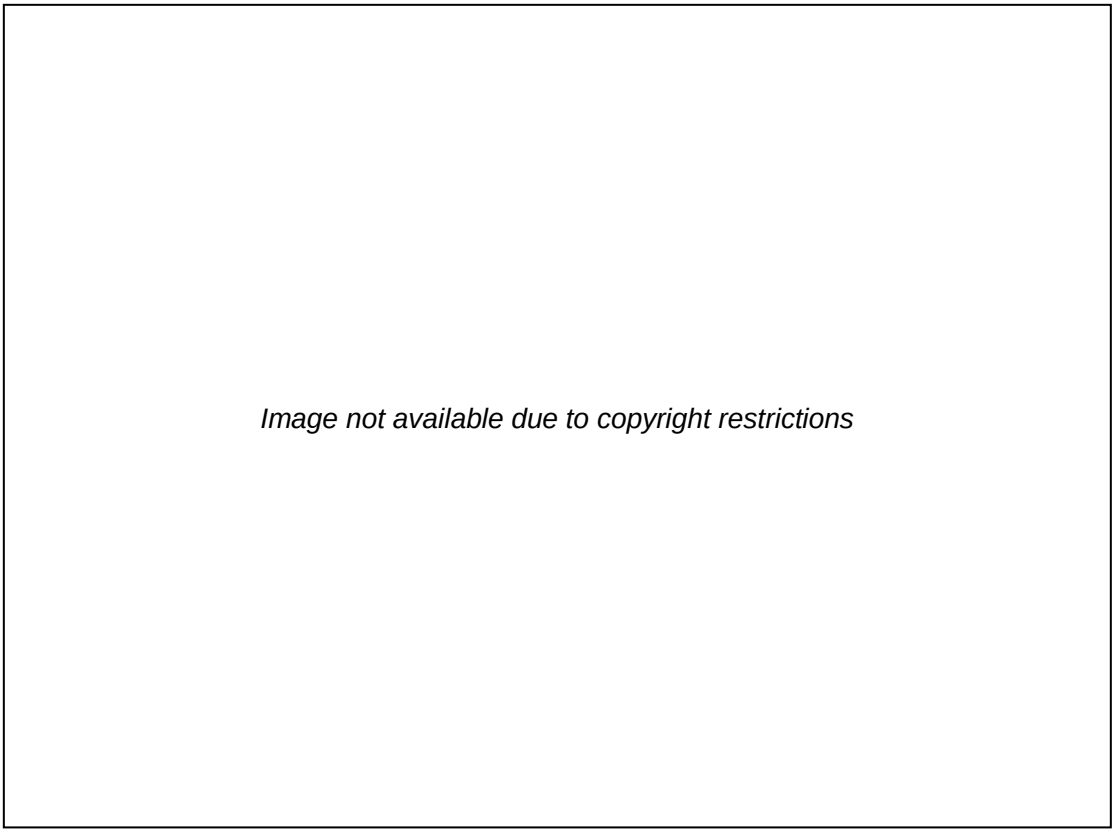
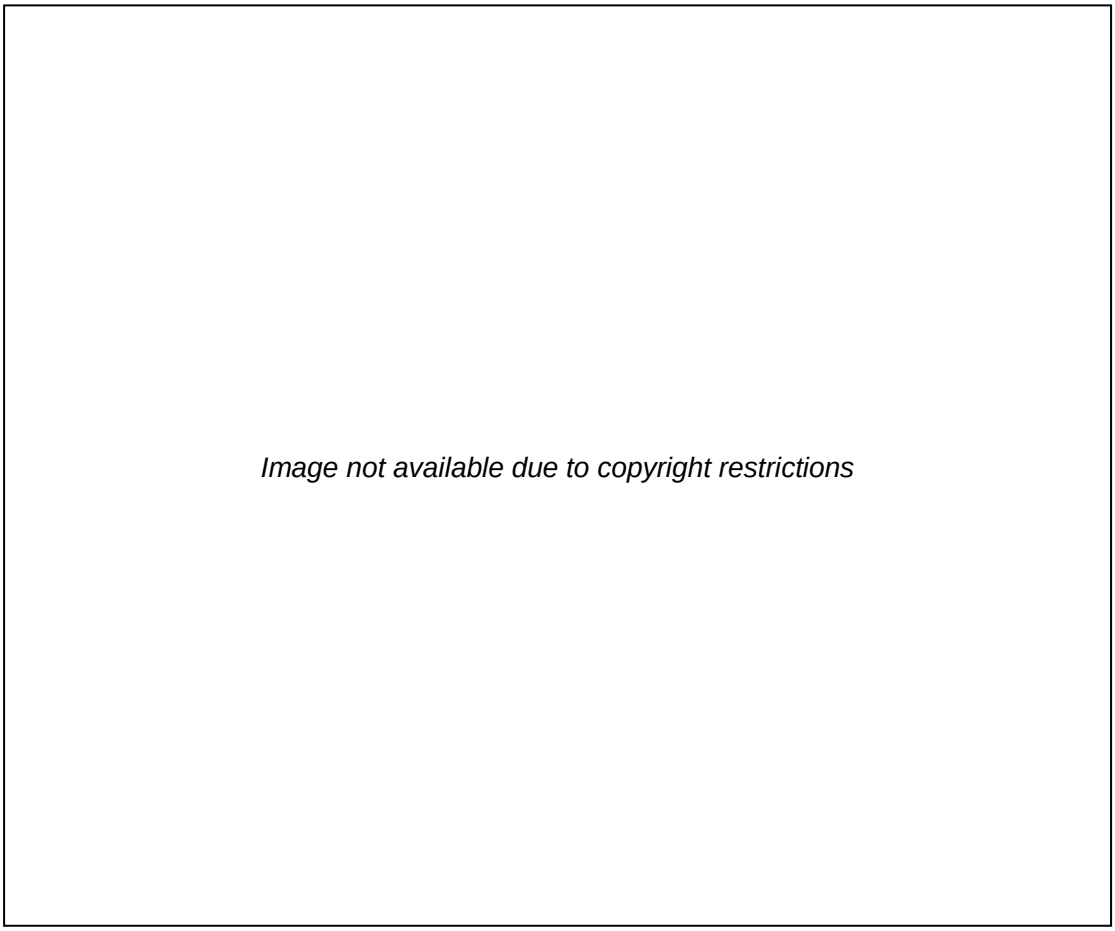


Figure 2.2.15 Global prevalence of HBV[480]



carriers develop serious liver-related diseases similar to those of HCV such as chronic hepatitis, cirrhosis and HCC, with HBV infection estimated to causes more than one million deaths each year[480-482]. Adults infected with HBV usually develop symptomatic acute hepatitis B and recover without intervention, with 5-10% developing the chronic carrier state[482]. Infected children, on the other hand rarely develop symptomatic acute infection, with 25-90% going on to become chronic carriers[482]. However, HBV is a vaccine-preventable disease, with a safe and effective vaccine available for over 20 years[482;483]. Unfortunately, worldwide infection persists and although global control is achievable with current technology, it is yet to be attained[482;483].

2.2.8.2 Treatment for HBV

Unlike HCV, HBV is a DNA polymerase virus that persists in the host nucleus as so called cccDNA[484]. This means that completely eradicating the infection is very difficult and consequently infection with HBV is a complicated and dynamic process[485]. The spectrum of disease and natural history of chronic HBV are diverse and variable, ranging from an inactive carrier state, which poses little risk to the carrier, to progressive chronic hepatitis B, which is responsible, via HBV-DNA replication, for the evolution of cirrhosis and HCC[485].

Fortunately, there are treatments available to control HBV infection[485]. Treatment is aimed at halting the progression of liver disease and improving the clinical outlook, while eliminating infectivity to prevent the transmission and spread of the virus[482;485;486]. These treatments are divided into two main classes, immune modulators which aim to help the immune system mount a defence against the virus, and antivirals which aim to suppress HBV by interfering with viral replication[482;485]. The drugs in both of these classes have been described in detail previously in this chapter. The immune modulators are interferons (Section 2.2.6) while the antivirals are tenofovir, emtricitabine and lamivudine, the same NRTIs used in the treatment of HIV (Section 2.1.9), plus telbivudine, entecavir and adefovir, which work in the same way[485]. Consequently, due to the dual activity of some ARVs against HIV and HBV, people coinfected with HIV/HBV do not have to make big changes to their treatment regimens. They are advised to take a cART regimen containing tenofovir, emtricitabine or lamivudine, plus a third agent active against HIV[193;485].

2.2.8.3 Dual hepatitis infection

HBV is thought to be about 100 times more infectious than HIV[486;487]. Transmission of HBV occurs in much the same way as HIV and HCV, via blood to blood contact. However, HBV is known to transmit more frequently than HCV during sexual contact[483;486]. HBsAg has been found in all bodily secretions excretions, but only blood, vaginal and menstrual fluids, semen and in those with very high viral loads, saliva, have been shown to be

infectious[486;487]. Due to the routes of infection, infants born to infected mothers, injecting drug users sharing equipment, sexually active individuals (both heterosexuals and MSM) and those providing or receiving acupuncture or tattooing are all thought to be at high risk of contracting HBV[486;487].

As a consequence of shared transmission routes, the likelihood of being exposed to both HCV and HBV is high, particularly in areas with a high prevalence of HBV and HCV, therefore dual infection with both viruses is common[488]. Overall, the prevalence of dual infection is around 10-20% in those with chronic HBV, and 2-10% in those HCVAb positive[488]. However, the prevalence can be far higher in at risk populations, such as IDUs in incarceration (43% with dual infection)[489], while in general the risk factors for dual infection are the same as for monoinfection with either virus[488].

Interference between the two viruses in a dually infected person has been well documented[488]. The titre of serum HBV-DNA in a person with actively replicating HCV RNA has been shown to be markedly lower than in a HBV monoinfected person[490;491]. Further, it has been suggested that HCV infection may suppress HBV completely and become the sole cause of persistent hepatitis and liver injury in some dually infected people[492]. Conversely, it has also been shown that HCV RNA levels are significantly decreased in persons with actively replicating HBV-DNA, compared to those HCV monoinfected, suggesting an inverse relationship between the replicative patterns of both viruses[490;493].

Despite the evidence to suggest that in many cases dual infection with HBV/HCV results in the suppression of one or the other virus, several studies have reported more severe clinical outcomes in these patients[488;494;495], with higher rates of decompensated liver disease found in dually infected patients, compared with those monoinfected with either virus[496]. Other studies have also shown more severe histological lesions in dual infection, including a higher prevalence of cirrhosis[493]. Further, the clinical impact of multiple infection has been shown to be worse than infection with either virus, with accelerated progression to HCC as HCV and HBV replicate in the same hepatocytes[497-499].

However, it remains that in the majority of dual infection cases HBV-DNA levels are low or undetectable and HCV is responsible for the activity of chronic hepatitis[499]. Consequently, dually infected patients should usually receive treatment for HCV[500], with SVR rates broadly comparable with HCV monoinfected individuals and those coinfected with HIV[501;502].

Chapter 3

Data and Methodology

3.1 Data

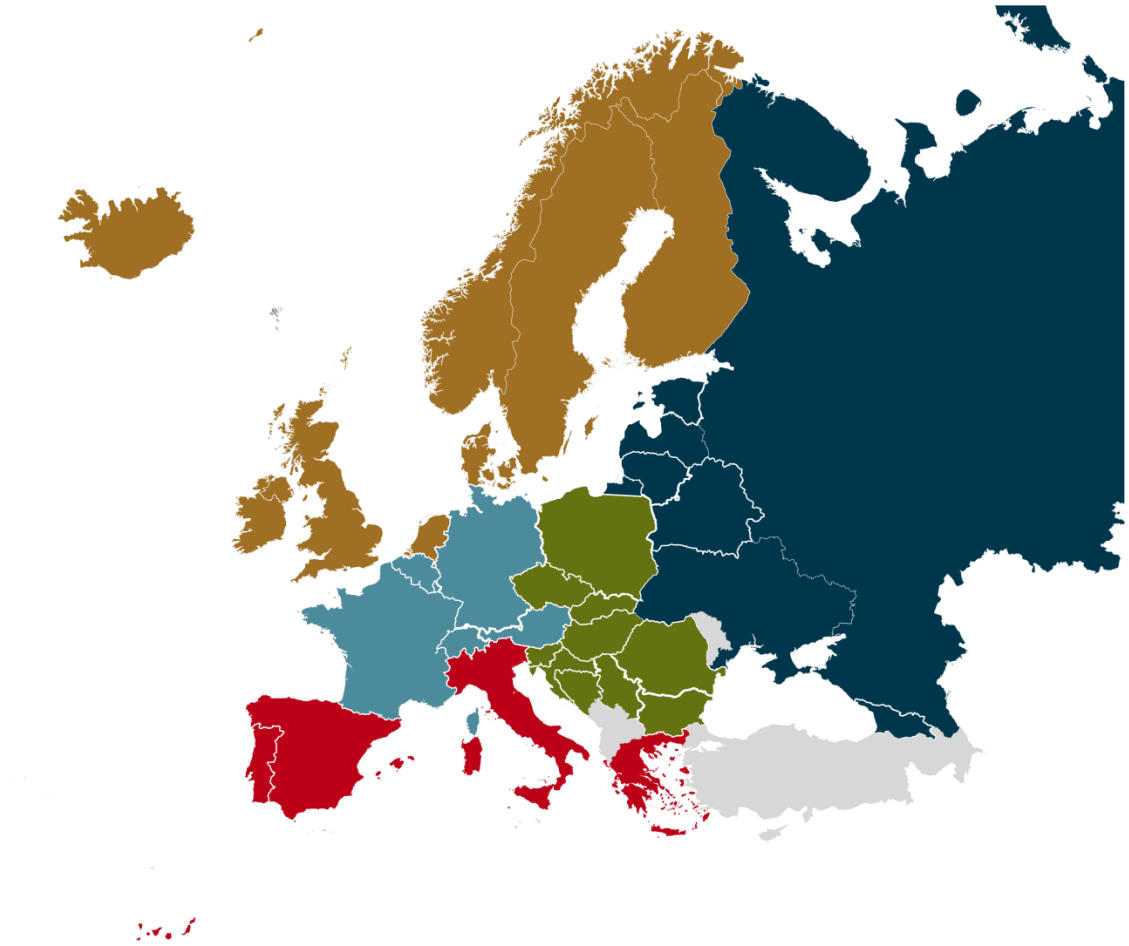
The data analysed in this thesis are predominantly from the EuroSIDA study group. EuroSIDA is a large European observational cohort study which is described in detail below. A small section of this thesis analyses data from the Swiss HIV Cohort Study (SHCS), to validate findings in EuroSIDA. The SHCS is introduced in Chapter 8 where it is analysed.

3.1.1 EuroSIDA

EuroSIDA is a large prospective observational cohort study, which at the time of the most recent data extraction in March 2015 included 18,795 individuals with HIV-1 infection and a total of 144,250 person years of follow-up. The study is one of the largest international cohort studies of HIV including 115 centres across 35 European countries, Israel and Argentina (Figure 3.1). EuroSIDA was initiated in May 1994 when it took up the work generated by its predecessor, the AIDS in Europe study[503]. AIDS in Europe was an early European study of HIV which collected data on every AIDS-diagnosed patient in participating European centres between 1979 and 1989. The study included 17 European countries and a total of 6,572 patients. Using patient case notes they collected data on demographics, HIV antibody status, CD4 lymphocyte counts, use of available ART, *pneumocystis jiroveci pneumonia* prophylaxis, and details of AIDS-defining illnesses.

AIDS in Europe was coordinated by a group of clinicians and scientists in Copenhagen led by Professor Jens Lundgren, director of the Copenhagen HIV Programme (CHIP). Professor Lundgren then established EuroSIDA upon the completion of AIDS in Europe with the aim of collecting detailed prospective data on treatment and illnesses associated with HIV. CHIP has since developed into a centre of excellence for the management and analysis of HIV cohort data. At the time of writing EuroSIDA have published 219 scientific papers in peer-reviewed journals and presented work at all major international conferences for many years. Further details of CHIP and the studies they coordinate can be found at www.chip.dk.

Figure 3.1 EuroSIDA countries and regions



EuroSIDA regions: South, North, West Central, East Central, East and Argentina

Primary support for EuroSIDA is provided by the European Commission BIOMED 1 (CT94-1637), BIOMED 2 (CT97-2713), the 5th Framework (QLK2-2000-00773), the 6th Framework (LSHP-CT-2006-018632), and the 7th Framework (FP7/2007-2013, EuroCoord n° 260694) programmes. Current support also includes unrestricted grants by Janssen R&D, Merck and Co. Inc., Pfizer Inc., GlaxoSmithKline LLC. In addition, the participation of centres from Switzerland was supported by The Swiss National Science Foundation (Grant 108787).

EuroSIDA is guided by a steering committee of scientists and clinicians with representatives from around Europe. Professor Jürgen Rockstroh from Bonn, Germany, is the current chair of the steering committee. Positions on the steering committee are regularly rotated and decided by nominations and elections. Appendix I provides a full list of the current members of the EuroSIDA study group.

EuroSIDA has developed a robust review process for the consideration of new research proposals. A summary of a potential project's background, aims, feasibility, statistical methodology and cost is sent to the coordinating centre and then reviewed by two members of the steering committee. After any amendments are incorporated the proposal is introduced and discussed by the steering committee at the next scheduled steering committee meeting. If there are no further comments or recommendations the project is approved and work may begin. The template for new study proposals can be found at http://www.chip.dk/Ongoing-Studies/EuroSIDA/Study-documents.

EuroSIDA has close links to UCL and is supported by the Research Department of Infection and Population Health, which provides expert statistical advice and analysis. Genotypic resistance and subtype data are provided from the virology laboratory group originally based in London and since 2004 in Badalona, Spain. Co-ordinators of the statistical centre and the virology group are involved in the development of new proposals and analysis of EuroSIDA scientific projects and also have representation on the steering committee.

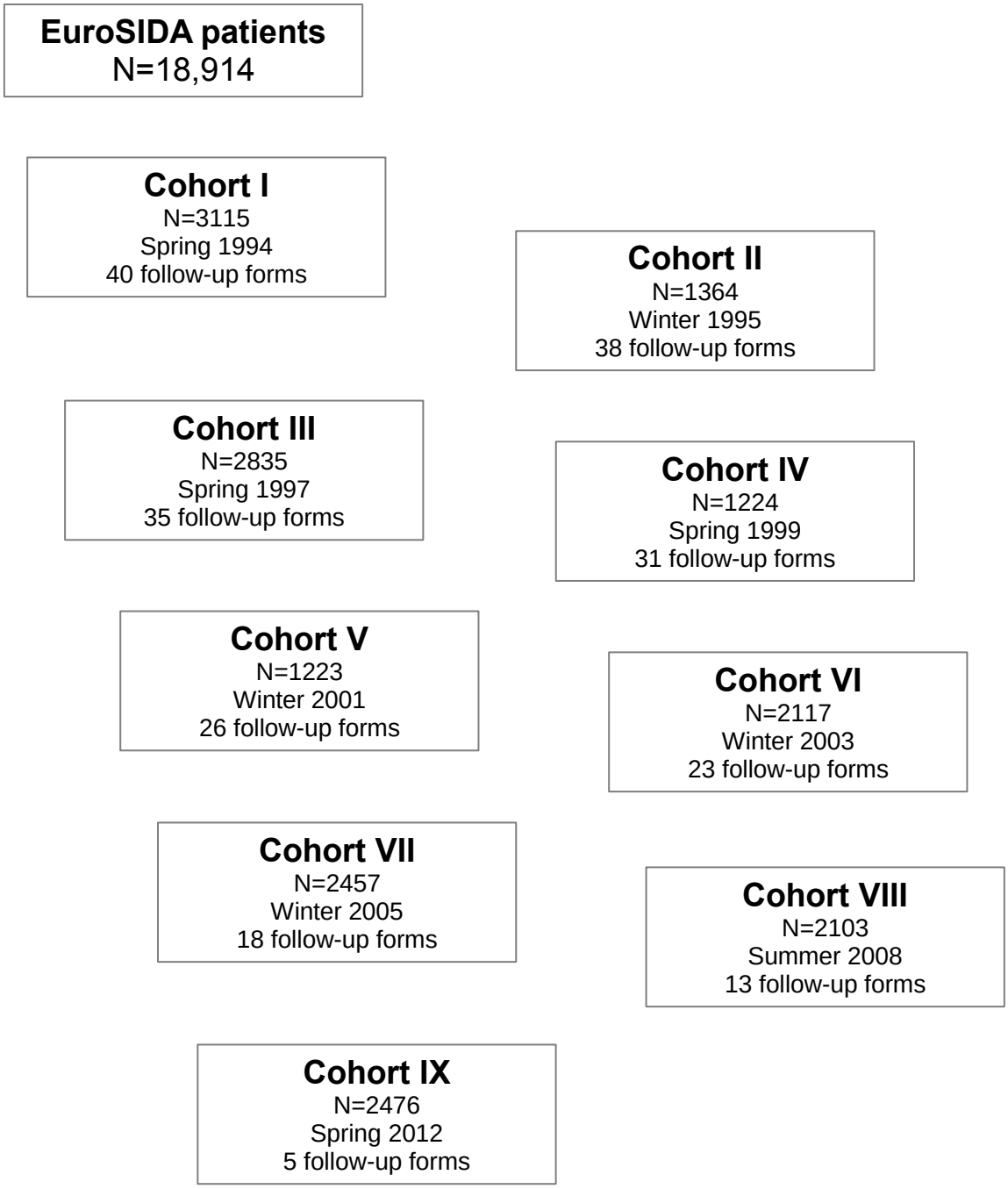
EuroSIDA also contributes data to the D:A:D study (Data collection on Adverse events of anti-HIV Drugs), COHERE (Collaboration of Observational HIV Epidemiological Research in Europe), and ART-CC (Antiviral Therapy Cohort Collaboration) as well as other ad hoc projects after approval by the steering committee[504-506].

3.1.2 Data collection

EuroSIDA have so far enrolled nine cohorts of consecutive HIV-positive individuals aged 16 or over. Data are collected at the individual clinical centres by health care providers. Individuals with a routine, booked clinic outpatient appointment are enrolled consecutively to ensure that they represent an unbiased selection of those under clinical care at each clinical site. During each enrolment period, sites enrol until a predefined cap for each region/clinic is reached. A summary of each cohort and the corresponding date of inception is given in Figure 3.2.

For the first three cohorts eligible individuals must have had a CD4 lymphocyte count of <500cells/mm^3 in the four months prior to entry. This criterion was removed for the following cohorts. From cohort VI onwards, in order to increase the number of individuals from Eastern Europe, half of the total number of individuals enrolled have been from Eastern European countries. This was introduced so that a more comprehensive view of the HIV epidemic in Eastern Europe could be described.

Figure 3.2 Total patients, enrolment dates and number of follow-up forms available according to cohort in EuroSIDA



Data is collected at sites during routine visits to access clinical care on a standardised data collection form; the data are collected at baseline and collated every six months thereafter. The EuroSIDA enrolment and follow-up forms can be found at http://www.chip.dk/Ongoing-Studies/EuroSIDA/Study-documents. The current working EuroSIDA database compiled in March 2015 contains follow-up to November 2013 (median date of last visit). At each

follow-up visit all CD4 cell counts and viral load measures taken since last follow-up are collected. A total of 507,418 CD4 cell counts from 18,724 patients (median 21 (interquartile range (IQR) 9-41) per patient) and 444,891 viral load measurements from 16,773 patients (median 21 (IQR 10-40) per patient) have been collected so far.

Dates of starting and stopping each antiretroviral drug, reasons for stopping each drug and the use of prophylaxis against opportunistic infections are also collected. All dates of diagnosis of AIDS-defining illnesses are collected using the 1993 CDC definition of AIDS[507]. Dosing levels of drugs are not collected in EuroSIDA, however, an assumption is made that if individuals start ritonavir (RTV) plus another PI at the same time, this is a boosted PI regimen with low dose RTV.

EuroSIDA has been quick to react to the changing nature of the HIV epidemic over time. In 2001 after the introduction of effective treatment for HIV in the mid-1990s and the subsequent reduction in AIDS-related mortality, focus shifted towards collecting data on non-AIDS defining illnesses and causes of death. In 2006 as focus shifted to coinfection with hepatitis, data collection forms were updated to include a host of hepatitis antibody and viral load tests, which will be discussed in detail shortly.

3.1.2.1 Plasma sample repository

Since 1997 EuroSIDA has also requested that plasma samples are collected from participants every six months. These samples are then transferred for storage in the central repository at the Copenhagen coordinating centre. Samples are stored at -80 degrees Celsius, which prevents degradation of the material and ensures their long term viability. The repository currently holds 118,614 plasma samples from 11,150 patients, with a median of 9 (IQR 4-18) samples available per patient. Samples may then be selected based on a number of criteria to be involved in on-going projects. Projects looking at HIV resistance mutations have used samples to extract HIV RNA for sequence analysis whereby genotypic resistance mutations can be identified. Other projects looking at coinfection with HCV have used the samples to extract HCV antibodies and HCV RNA viral loads to classify the status of HCV infection.

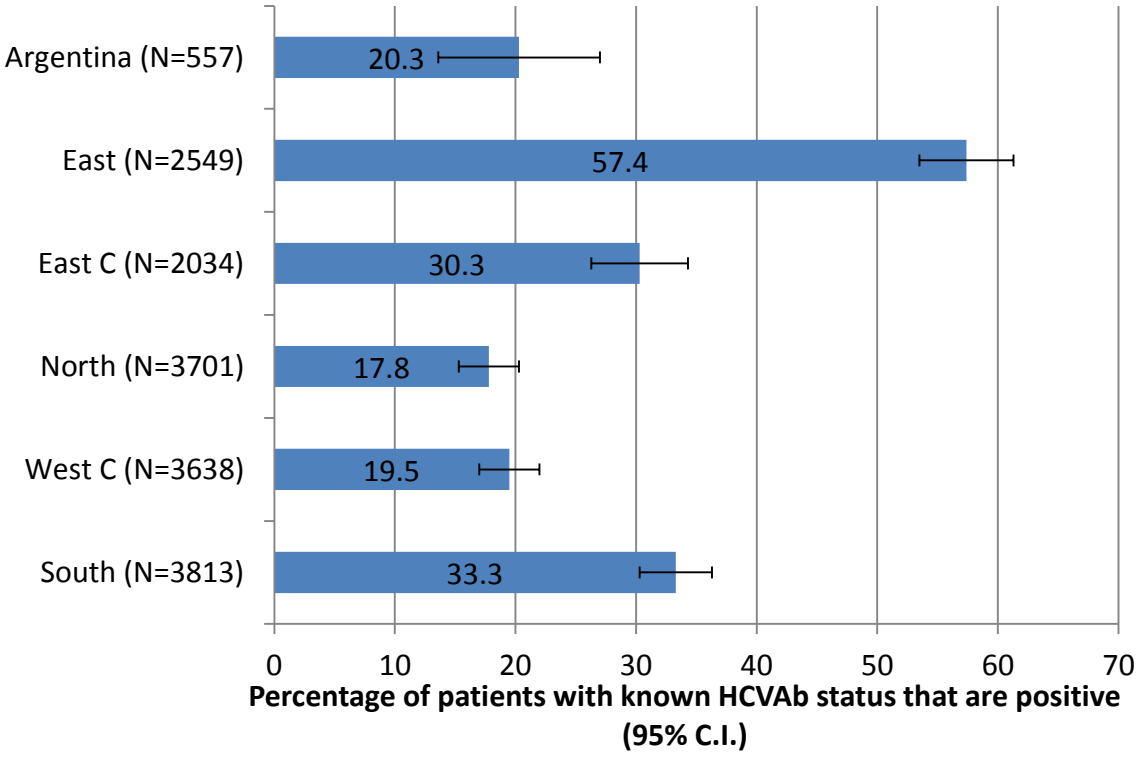
Although samples are collected during prospective follow-up, analysis of the samples is undertaken retrospectively, therefore, the results of any analysis performed on the samples are not routinely passed on to the health care providers that obtained the samples.

3.1.2.2 EuroSIDA Hepatitis data

EuroSIDA now collects a variety of information on hepatitis coinfection. Data on anti-HCV antibodies (HCVAb) have been collected on the standard EuroSIDA follow-up form since 1997. Individuals that died or were lost to follow-up prior to this date did not routinely have HCVAb data collected. Clinical sites that have measured HCV RNA are requested to report the measurement to EuroSIDA along with HCV genotype if genotyping has been performed. In 2006 all EuroSIDA participants who had unknown HCVAb status were tested using stored plasma samples, where available, and those found to be HCVAb positive were then tested for HCV RNA and HCV genotype accordingly. Further, any individuals that were known to be HCVAb positive but were missing HCV RNA data were identified and where stored plasma was available, tested for HCV RNA and HCV genotype. Consequently, EuroSIDA now has a population of over 5,000 well-defined HCV coinfected individuals, with estimated coinfection rates ranging from 17.8% in the North to 57.4% in the East, based on the current dataset (Figure 3.3).

Data on HBV coinfection has also been collected since 2006. EuroSIDA collect HBV antibodies, HBV DNA and hepatitis B surface antigen (HBsAg) which allows for an accurate description of each individual's HBV infection status. In 2006 all HBsAg positive individuals were additionally tested for hepatitis D virus (HDV) antibodies, where stored plasma

Figure 3.3 Estimated prevalence of HCVAb by region of EuroSIDA



samples were available. Treatment start and stop dates for both HCV and HBV therapy are collected in EuroSIDA as part of the main follow-up form.

EuroSIDA has the ability to estimate liver fibrosis levels using surrogate markers such as the APRI score[425]. These markers are calculated based on an individual's age and lab parameters such as ALT, AST and platelet counts and are described in detail in Section 2.2.5.3 of the introduction to this thesis. However, recently EuroSIDA have also added other markers of liver fibrosis. Since 2010, clinical sites that perform liver biopsy or Fibroscan® elastography are required to report that information to the coordinating centre[421]. Further, in 2008, using the plasma sample repository data, the biomarker hyaluronic acid was determined for all coinfected patients, where plasma samples were available.

3.1.2.3 Monitoring and causes of death

EuroSIDA coordinating centre members visit all clinical sites participating in EuroSIDA to monitor patient selection criteria and to quality check the data supplied. EuroSIDA monitors check information in the EuroSIDA database against individual case notes for all individuals that experience a clinical event and a randomly selected 10% of all other patients per year. Further, occasionally monitoring is used to gather extra information from case notes that may have been missed off the original data submission. All EuroSIDA centres have ethical approval from their own local and national authorities. However, the contract EuroSIDA has with its funders, the European Commission, requires that EuroSIDA retain copies of each centre's ethnical approval forms at the coordinating centre. The data collected in EuroSIDA are summarised in Table 3.1.

Deaths and causes of death are also captured in EuroSIDA. Traditionally deaths in HIV-positive individuals were coded using the international classification of diseases system (ICD), which classified deaths as HIV-related or non-HIV-related[508]. However, after the introduction of cART in 1996, the rate of AIDS-related death began to reduce over time. Consequently, the number of deaths attributed to non-AIDS-related causes, such as malignancies, liver-related death and cardiovascular disease, began to rise[509;510]. Therefore, in order to accurately monitor causes of death among HIV-positive individuals a new system of coding was required.

In 2004 a group of European researchers, including the EuroSIDA study group, started a project to create a uniform classification system for coding deaths among HIV-positive individuals. The coding of causes of death in HIV (CoDe) classification system involves the collection of a wide range of data from individual demographics, potential risk factors for

death, treatment history, laboratory measurements, autopsy results and the potential role of toxicities in relation to death in order to determine the underlying cause of death[511;512]. The 4-page CoDe form which is filled out for each death in EuroSIDA can be seen in Appendix II.

Completed CoDe forms are reviewed by clinicians at CHIP. In most cases the cause of death is obvious. However, more complicated cases, which usually involve competing risks of death, are discussed by a panel of clinicians at CHIP. If the CHIP clinicians cannot come to an agreement about the cause of death then the case will be referred to an external reviewer. In instances where the external reviewer and clinicians at CHIP disagree on the underlying cause of death they are both asked to reconsider. If agreement still cannot be reached then a third expert reviewer is asked to make the tie-breaking decision[511;512]. The classification system for underlying cause of death is displayed in Table 3.2.

For historical data, when a CoDe form has not been completed or insufficient information is available, EuroSIDA also have a process for determining whether each cause of death is classified as AIDS-related or non-AIDS related. If AIDS-defining events are diagnosed and reported then the proximity of these events to the time of death is used to determine whether the death was AIDS-related[511;512]. Deaths are classified as AIDS-related if the survival time following diagnosis with the underlying cause of death is lower than the upper quartile of the of survival time for that specific condition. For conditions where the survival time distribution is unknown a survival time of less than 17 or 12 months is used to determine whether the death was AIDS-related or not[511;512].

Table 3.1 Outline of EuroSIDA data

Demographics	**Hepatitis virology/serology results and dates*:**
Date of birth	HBV antibody
Gender	HBsAg
Mode of HIV infection	HBV DNA
Race	HCV antibody
Basic clinical information	HCV RNA
Height	HCV genotype
Weight	**Liver fibrosis parameters**
Blood pressure	Liver biopsy
Smoking	Fibroscan® elastography
Family history of MI	Hyaluronic acid
Pregnancy in women	APRI (calculation)
Active injecting drug use	FIB-4 (calculation)
Alcohol abuse	**Treatment for HBV and HCV infection:**
Clinical events	Start and stop dates
Diagnosed since last follow-up (with date of diagnosis):	**Treatment against infections**
Cardiovascular events	Drugs to prevent or treat opportunistic infection:
Metabolic events	Start and stop dates
Other organ events	**Treatment related to risk of cardiovascular disease**
Laboratory values (and dates of measurement)	Medication related to risk of cardiovascular disease:
Serum total and HDL cholesterol	Starting and stopping dates
Serum triglycerides	**Severe opportunistic infections**
Plasma glucose	Dates and diagnosis (definitive, presumptive, autopsy)
S-creatinine	**Other severe infections**
Haemoglobin	Dates and diagnosis (definitive, presumptive, autopsy)
Platelet count	**AIDS defining malignancies**
ALT	Dates and diagnosis (definitive, presumptive, autopsy)
AST	**Non-AIDS defining cancers**
INR	Dates and diagnosis (definitive, presumptive, autopsy)
Bilirubin	**For patients who died**
S-lactate (not LDH)	Date of death
S-amylase	Presumed cause
CD4 counts	CoDe case report form including autopsy report
HIV-RNA	
HIV subtyping	
Resistance testing	
Antiretroviral treatment	
History of antiretrovirals taken:	
Starting and stopping dates	
If discontinued, reason for discontinuation	
Adherence rating	

***HBV genotype and HDV antibodies are also tested from the central plasma repository**

Table 3.2 Causes of death classification from CoDe methodology

Code	Cause of Death
1	AIDS (ongoing active disease)
1.1	Infection
1.2	Malignancy
2	Infection (other than 01.1)
2.1	Bacterial
02.1.1	Bacterial with sepsis
2.2	Others
02.2.1	Others with sepsis
2.3	Unknown aetiology
02.3.1	Unknown with sepsis
3	Chronic viral hepatitis (progression of/complication to)
3.1	HCV
03.1.1	HCV with cirrhosis
03.1.2	HCV with liver failure
3.2	HBV
03.2.1	HBV with cirrhosis
03.2.2	HBV with liver failure
4	Malignancy (other than 01.2 and 03, 03.1, 03.2)
4.03	ANUS - Anal cancer
4.04	BLAD - Bladder cancer
4.05	BONE - Bone cancer
4.06	BRAC - Brain cancer
4.07	BRCA - Breast cancer
04.10.1	ALL - Leukaemia: Acute lymphoid
04.10.2	AML - Leukaemia: Acute myeloid
04.10.3	CLL - Leukaemia: Chronic lymphoid
04.10.4	CML - Leukaemia: Chronic myeloid
04.10.9	LEUK - Leukaemia: unspecified
4.18	COLO - Colon cancer
4.11	COTC - Connective tissue cancer
4.12	ESOP - Esophagus cancer
4.13	GALL - Gallbladder cancer
4.14	GYCA - Gynaecologic cancer
4.15	HDL - Hodgkin lymphoma
4.16	HENE - Head and neck (incl. face) cancers
4.17	KIDN - Kidney cancer
4.19	LIPC - Lip cancer
4.2	LIVR - Liver cancer
4.21	LUNG - Lung cancer
4.22	MALM - Malignant melanoma
4.27	MULM - Multiple myeloma
4.29	PANC - Pancreas cancer
4.31	PENC - Penile cancer
4.32	PROS - Prostate cancer
4.33	RECT - Rectum cancer

4.34	STOM - Stomach cancer
4.35	TESE - Testicular seminoma
4.36	UTER - Uterus cancer
04.40.1	MEAC - Metastasis: of adenocarcinoma
04.40.2	MEOC - Metastasis: of other cancer type
04.40.3	MESC - Metastasis: of squamuos cell carcinoma
04.40.9	META - Metastasis: unspecified
4.9	OTH - Other Malignancy Type
4.99	UNKP - Unknown Malignancy Type
5	Diabetes Mellitus (complication to)
6	Pancreatitis
7	Lactic acidosis
8	MI or other ischemic heart disease
8.1	AMI
08.1.1	Definitive AMI (Dundee 1)
08.1.2	Possible AMI (Dundee 2/9)
8.2	Other ischemic heart disease
9	Stroke
10	Gastro-intestinal haemorrhage (if chosen, specify underlying cause)
11	Primary pulmonary hypertension
12	Lung embolus
13	Chronic obstructive lung disease
14	Liver failure (other than 03, 03.1, 03.2)
15	Renal failure
16	Accident or other violent death (not suicide)
17	Suicide
18	Euthenasia
19	Substrance abuse (active)
19.1	Chronic Alcohol abuse
19.2	Chronic intravenous drug-use
19.3	Acute intoxication
20	Haematological disease (other causes)
21	Endocrine disease (other causes)
22	Psychiatric disease (other causes)
22.1	Mental and behavioural disorders due to use of psychoactive substances (other than alcohol and intravenous opioids)
22.2	Schizophrenia, schizotypal and delusional Disorders
22.3	Mood /Affective disorders (Major depressive disorder, Bipolar disorder and other mood disorders)
22.4	Neurotic, stress-related and somatoform disorders (including anxiety disorders, phobias, OCD, stress reaction, dissociative disorders, somatoform disorders)
22.5	Behavioral syndromes associated with physiological disturbances and physical factors (including eating disorders, sleep disorders, sexual disorders)
22.9	Other psychiatric disorders
23	CNS disease (other causes)
23.1	Movement disorders (Parkinson’s disease; dystonias and Parkinson-like syndromes)

23.2	Degenerative disorders of the central nervous system (Alzheimer's disease; Creutzfeldt–Jakob disease and other degenerative diseases of nervous system)
23.3	Demyelinating diseases of the central nervous system (Multiple sclerosis, other demyelinating diseases)
23.4	Epilepsy (including localised and generalized epilepsy and epileptic syndromes)
23.5	Polyneuropathies (Guillain–Barré syndrome and other polyneuropathies/disorders of the peripheral nervous system)
23.6	Diseases of myoneural junction and muscle (Miastenia gravis and other myoneural disorders)
23.9	Other disorders of the nervous system
24	Heart or vascular (other causes)
25	Respiratory disease (other causes)
26	Digestive system disease (other causes)
27	Skin and motor system disease (other causes)
28	Urogential disease (other causes)
29	Obstetric complications
30	Congenital disorders
31	Symptoms caused by mitochondrial toxicity (other than 06, 07)
32	Bleeding (haemophilia)
33	Sudden infant death
33.1	Child abuse
90	Other causes
91	Unclassifiable causes
92	Unknown
92.1	Unknown, Competing risks

3.2 Methodology

Many statistical methods have been used to analyse the data in this thesis. An introductory statistical methods section at the start of each chapter will describe which methods were used to perform the analysis along with inclusion and exclusion criteria, while they are also summarised here in Table 3.3. The remainder of this chapter will provide an overview of the statistical methods used throughout this thesis. All statistical analyses have been performed using SAS version 9.2 or above (SAS institute, Cary, North Carolina, USA).

3.2.1 Summary statistics

It is important when analysing data to provide summary statistics that give an overview of the population being studied. These initial analyses can help to find any obvious data errors that need to be amended and can be useful to compare different study populations before any formal analysis is performed. This is an important step in cohort study analysis as there will often be differences between the groups of patients being studied due to the absence of patient randomisation seen in clinical trials, as discussed in more detail below.

Throughout this thesis, for categorical variables the total number of patients (N) and the percentage (%) in each category will be reported. For continuous variables the mean and standard deviation (SD) are presented when the data are normally distributed, while the median and interquartile range (IQR) are presented when the data are skewed. When data are approximately normally distributed the mean and median will be similar but they can be quite different from each other when data are highly skewed.

When comparing categorical characteristics of different groups of patients the Pearson's chi-square test is used. In cases where there are fewer than 5 patients in any category then Fisher's exact test will be used. When continuous data are normally distributed unpaired t-tests or f-tests will be used to compare different groups, while when comparing more than 1 set of values between the same patients, HIV viral load at 2 separate time points for example, a paired t-test may be used. When comparing 2 groups of continuous data that are skewed the Wilcoxon rank sum test is performed, while for skewed continuous data with more than 2 groups the Kruskal-Wallis test will be used, which is an extension of the Wilcoxon rank sum test. Both the Wilcoxon and Kruskal-Wallis tests are non-parametric and make no assumptions about the underlying distribution of the data.

All statistical tests are performed two-sided, unless stated otherwise, with a *P*-value less than 0.05 taken to be statistically significant, meaning there is sufficient evidence to

Table 3.3 Summary of data for each analysis

Chapter	*EuroSIDA dataset*	*N in dataset*	*N included in analysis*	*Inclusion criteria*	*Study endpoints*	*Publication details (Date accepted)*
4	D35	16,594	1,541	All chronically infected HIV/HCV coinfected individuals positive for both HCVAb and HCV RNA	HCV RNA profiles	HIV Medicine (February 2013)
5	D35	16,594	1,984	All chronically infected HIV/HCV coinfected individuals positive for both HCVAb and HCV RNA	Treatment for HCV with interferon-based therapy	HIV Medicine (May 2013)
6	D37	18,913	9,535	All EuroSIDA participants to have initiated cART with known HCVAb status	Discontinuation of ART components	AIDS (September 2013)
7	D38	18,786	3,987	All HCVAb positive individuals with follow-up available after 1/1/2000	Liver-related death	AIDS (February 2015)
8	D40	18,914	4,011	All HCVAb positive individuals with follow-up available after 1/1/2000	Liver-related death prognostic score	Submission to CID planned in June 2015

conclude that the null hypothesis of no difference between the groups can be rejected. The clinical significance of all findings reported in this thesis will also be considered looking at the size of the effect and the width of the confidence intervals.

3.2.2 Statistical models

Statistical models are able to describe the relationship between variables of interest and also act as a predictive tool. They quantify the relationship between an outcome and explanatory variables, while incorporating a random element to account for the nature of chance as observed data deviate from predicted outcomes. Importantly, statistical modelling allows us to control for confounding. Confounding is the process by which a spurious relationship may be found between an outcome and explanatory variable via a correlated third variable. If a variable is correlated with the outcome of interest and the explanatory variable then confounding may occur. For example, a group of men over the age of 50 would be more likely to develop certain cancers and non-AIDS defining illnesses than a group of women below the age of 30. If age was not adjusted for in the analysis then it may be possible to come to the conclusion that men are more likely to develop some cancers and non-AIDS defining conditions than women. In this scenario it is said that age is confounding the relationship between sex and development of a non-AIDS-defining illness.

In randomised clinical trials confounding is avoided because participants are randomised to each intervention arm. Therefore, in a large enough sample size, participant characteristics will be evenly balanced between intervention arms. However, in observational studies there is no randomisation of participants or allocation of interventions, so it is likely that there will be significant imbalances in the study population that may lead to spurious inference. Fortunately, the effect of measured and known confounding can be adjusted for by using multivariable models, assessing the impact of more than one explanatory variable including potential confounders, on an outcome of interest. Including potential confounding variables in the model means that *adjusted* estimates are calculated which can account for the role of confounding by variables included in the model. The difference between estimates from univariable analysis and multivariable analysis then describes the magnitude of the confounding effect. However, regardless of the steps taken to address confounding there will always be a level of unmeasured confounding in observational studies that cannot be adjusted for in the analysis.

In some instances a factor may have a different effect on an outcome depending on the level of the factor. For example, HIV/HCV coinfected individuals aged 40 or below with a CD4 cell count of 500cells/mm^3 may be less likely to have liver fibrosis than similar individuals over the age of 60. This describes an interaction between CD4 cell count and

age. Interaction terms may also be adjusted for in multivariable models; however, in a model with many explanatory variables there are many potential interaction terms. Therefore, interactions are not routinely tested in multivariable modelling as it will increase the risk of a false positive result due to repeated statistical testing. Instead, interactions which are of potential interest will selected *a priori* for testing based on empirical knowledge of the subject area.

There are four main methods of statistical analysis used in this thesis: logistic regression, Cox proportional hazards regression, Poisson regression and mixed effects modelling. An overview of each of these methods, including in which scenarios they are used and how they are interpreted is given below.

3.2.2.1 Logistic regression

Logistic regression models are used when the outcome of interest is a binary variable which takes the form of a success or failure. For example, when modelling death, for each individual in the analysis the outcome of interest will be whether they have died or not, there are only two possible outcomes. In a simple analysis the proportion of individuals that have died could be compared between different groups, males and females for example. However, when using multivariable modelling to account for confounding or statistical interactions the binary outcome has to be transformed so that it takes values from $-\infty$ to ∞ and can be treated as a continuous stochastic variable[513;514]. Logistic regression takes its name from the fact that the transformation used is called the *logit* function, shown below in Equation 3.1, where p is the proportion of successes from the binary outcome variable.

Equation 3.1 The logit function

$$Logit(p) = \frac{log(p)}{log(1-p)}$$

The logistic regression model then takes the form shown in Equation 3.2, where β_n are the estimated linear regression coefficients and x_n are the explanatory variables of interest. The regression coefficients then describe the effect of each explanatory variable on the outcome independent of, or adjusted for, the other explanatory variables.

Equation 3.2 The logistic regression model

$$Logit(p) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_n x_n$$

The logistic regression model has a number of desirable properties. The *logit* function is essentially the *log* of the odds of an event, which is the number of successes divided by the number of failures. When back-transforming the linear regression coefficient estimates

each coefficient is identical to the estimated odds ratio of association between x_i and the outcome[513;514]. Therefore, logistic regression can be used to estimate the odds (and 95% C.I.) of an outcome given a set of explanatory variables. If the outcome is death and the explanatory variable of interest is sex then it would be possible to say that the odds of death are higher or lower for males compared to females. An odds ratio greater than one indicates increased odds of an outcome occurring, while an odds ratio below one, but greater than zero, indicates reduced odds of an outcome occurring. Consequently, a confidence interval that does not include one indicates a statistically significant finding.

3.2.2.2 Time to event analysis

Time to event analysis is used to study deviations in the time from a well-defined baseline common to all individuals to an event of interest. This is particularly common in the cohort study setting as it is often interesting to study time to death following the allocation of a treatment. In this case, when death is the outcome of interest, the method is referred to as survival analysis, as individual survival times are compared. Time to event analysis adds an extra level of information to simple logistic regression as it focuses on the time to an event rather than just whether the event occurred or not.

An interesting and useful feature of time to event analysis is that it can still be used when individuals are lost to follow-up or drop out of the analysis. When an individual becomes lost to follow-up their survival time is capped at the last known time of contact, which is known as right censoring. The individual may have died after the last point of contact but that information is not observed in the study. Individuals can also be left censored, which is where information is available on an individual prior to the baseline date; however this follow-up is capped at baseline to ensure the baseline time point remains common to all individuals. In EuroSIDA left censoring is common as information is often available from case notes on some individuals prior to enrolment in the study. Including information from before recruitment may introduce survivor bias where only those well enough to survive long enough to enter the study are included.

Censoring is an important element of time to event analysis as it allows for the inclusion of incomplete data. If data were only included for individuals with complete data, those with known baseline and last visit, then bias would be introduced to the analysis and potentially return spurious conclusions. Time to event analysis treats censoring as a random event which is not associated with the actual true survival time. Therefore, asymptotically censoring does not bias the results[513;514].

Kaplan-Meier estimation

Survival times from time to event analysis can be summarised by a mathematical function known as the survivor function. The survivor function describes the probability of survival at time t and is shown in Equation 3.3 below, where $F(t)$ is the cumulative probability of failure at time t.

Equation 3.3 The survivor function

$$S(t) = 1 - F(t)$$

The probability of survival at time t can be estimated using the non-parametric Kaplan-Meier estimator. The Kaplan-Meier estimator is shown in Equation 3.4 below, where n_i is the number of individuals at risk of death and d_i is the cumulative number of deaths at time t_i.

Equation 3.4 The Kaplan-Meier estimator function

$$\hat{S}(t) = \prod_{t_i<t} \frac{n_i - d_i}{n_i}$$

Kaplan-Meier estimation can be used to plot the survivor function and estimate the probability of survival, or failure at a given time of interest. The probability of failure is simply one minus the probability of survival. Kaplan-Meier estimation is used to compare the survival function of categorical or grouping variables without adjusting for other variables. The difference between the survival functions can then be tested statistically, using the log-rank non-parametric test, to see if one group has a higher probability of success or failure over the follow-up period[513;514].

Poisson regression

Poisson regression is a form of the generalised linear model where the errors, or differences between the observed and predicted values, belong to the Poisson distribution[513;514]. The formation of the Poisson regression model is similar to logistic regression except that it is used for time to event data and the outcome is the incidence rate of an event rather than the odds of an event. The incidence rate of an event is defined in Equation 3.5.

Equation 3.5 The incidence rate

$$Incidence\ rate = \frac{Number\ of\ events}{Total\ person\ years\ follow\ up}$$

The Poisson regression model then takes the form shown in Equation 3.6, where r is the incidence rate, β_n are the estimated linear regression coefficients and x_n are the explanatory variables of interest. Notice that as the outcome is now a rate and not a binary outcome the link function becomes the *log* transformation.

Equation 3.6 The Poisson regression model

$$Log(r) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_n x_n$$

Like logistic regression, Poisson regression also has desirable properties. When back-transforming the estimated linear regression coefficients using the *exponential* function each coefficient is identical to the estimated incidence rate ratio of association between x_i and the outcome, also known as the risk ratio[513;514]. Therefore, Poisson regression can be used to estimate the risk (and 95% C.I.) of an outcome given a set of explanatory variables. If the outcome is death and the explanatory variable of interest is sex then it would be possible to say that the risk of death is higher or lower for males compared to females. A risk ratio greater than one indicates increased risk of an outcome occurring, while a risk ratio below one, but greater than zero, indicates a reduced risk of an outcome occurring. Consequently, a confidence interval that does not include one indicates a statistically significant finding.

In Chapter 6 of this thesis generalised estimating equations are used to fit the parameters of the Poisson regression model. Generalised estimating equations are used to take account of within individual variability when each individual may contribute multiple endpoints[515].

Cox proportional hazards regression

Cox proportional hazards regression is one of the most common ways to analyse time to event data. The model is defined in much the same way as logistic regression and Poisson regression except that the outcome is defined as the hazard function[513;514;516]. The hazard function is shown in Equation 3.7, where $f(t)$ is the density function of survival times and $S(t)$ is the survivor function, as mentioned previously.

Equation 3.7 The hazard function

$$h(t) = \frac{f(t)}{S(t)}$$

The Cox proportional hazards model then takes the form shown in Equation 3.8, where β_n are the estimated linear regression coefficients and x_n are the explanatory variables of

interest. Notice that the intercept term β_0 is missing and replaced by $\log(h_0(t))$, which is the baseline hazard where all explanatory variables are set to zero. Cox proportional hazards models take their name from this set up as it assumes that the ratio of the hazards comparing different explanatory variables remains constant over time, which is known as the proportional hazards assumption.

Equation 3.8 The Cox proportional hazards model

$$Log(h(t)) = \log(h_0(t)) + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_n x_n$$

Although calculating $f(t)$, the density function of survival times, is not trivial, the proportional hazards assumption has the desirable property that when back-transforming using the *exponential* function, $h_0(t)$ cancels out so that calculation of $f(t)$ is not necessary[513;514;516]. This means that, in much the same way as Poisson regression, each coefficient is identical to the estimated hazard ratio of association between x_i and the outcome. Therefore, Cox proportional hazards regression can be used to estimate the hazard ratio (and 95% C.I.) of an outcome given a set of explanatory variables. A hazard ratio greater than one indicates increased risk of an outcome occurring, while a hazard ratio below one, but greater than zero, indicates a reduced risk of an outcome occurring. Consequently, a confidence interval that does not include one indicates a statistically significant finding.

Poisson regression and Cox proportional hazards models have many similarities; they are often used in similar situations and under certain circumstances produce the same results. The key difference between the two is that in Poisson regression the outcome of interest is a count of events, or a rate of events in a given period of follow-up, whereas in Cox models the outcome is the time to an event. Cox modelling also allows for censoring, where actual follow-up times may be unknown or unobserved. In this regard it is sometimes suggested that Poisson regression has less complicated inference, whereas Cox models are able to handle more complicated data structures.

3.2.2.3 Competing risk in time to event analysis

In some time to event analysis settings it is common for the outcome of interest to go unseen due to the occurrence of another event. For example, when studying liver-related death in HIV/HCV coinfection, individuals that could progress to liver-related death may die of an AIDS-defining illness in the meantime. These causes of death other than the outcome of interest are known as competing risks, events that prevent the outcome of interest from occurring. Competing risks are different from censoring, which prevents the event from being captured in the data. However, censoring is assumed to occur independently of the

outcome of interest and if censoring occurs systematically and is associated with the outcome of interest then it should also be considered a competing risk[517].

In the competing risks setting, when competing events are censored in the standard fashion, the Kaplan-Meier estimator is biased and may not accurately estimate the survival function[517]. Fortunately, a number of well-defined methods are available for handling competing risks analysis. The most widely used method in this setting is the Fine and Gray method[518;519]. In the Fine and Gray method, as the competing event prevents observation of the true survival time without the event of interest, all individuals that experience a competing event have their right censoring time replaced. Instead the event free survival time is estimated using the inverse probability of censoring. In other words, survival times are estimated as if the competing event had not occurred. This means that individuals are retained in the analysis population after a competing event even though they are not technically at risk of the event of interest. Modelling is then performed in the usual manner on this adjusted dataset with estimated true survival times. In Cox proportional hazards regression, this slightly abstract formulation leads to estimation of the sub-distribution hazard and hazard ratio, which is a good estimate of the true hazard ratio[518;519].

3.2.2.4 Mixed effects models

Mixed effects models, otherwise known as random effects models, are used in the analysis of repeated measurements within individuals. They are known as mixed effects models as they can include fixed and random effects. A fixed effect is a standard variable which is assumed to have a fixed effect on each individual in the analysis[520]. Examples of fixed effects include the effect of a treatment, age or gender, they are all assumed to have the same effect on an individual level. A random effect is a variable which represents a sample of a larger population, where the effect is variable at the individual level[520]. An example of a random effect would be clinical centre as the centres included in a study are a sample of those from the whole population. In a mixed effects model the effect of each centre is not fixed and is allowed to vary around a mean of zero. The mixed model takes the form shown in Equation 3.9, where y is a vector of observations for each individual, β is a vector of fixed effects, u is a vector of random effects, ϵ is a vector of random error terms and X and Z are matrices of regression coefficients.

Equation 3.9 The mixed effects model

$$y = X\beta + Zu + \epsilon$$

The inclusion of random effects, which vary for each individual, allows for the modelling of individual curves, or profiles, for each individual. If a random intercept term is included in

the model it allows the intercept to vary for each individual, while additionally including a random slope variable allows completely separate curves to be modelled for each individual, with different intercepts and slopes. The model then combines these individual curves to estimate an overall effect for each variable[520].

As mixed models analyse repeated measurements there is an additional level of variability to account for. Observations taken for one individual will be more similar than observations taken for another individual, while there may also be correlation between observations taken on one individual. For example, when analysing plasma HIV RNA levels over time in the same individual a high HIV RNA measurement would be more likely to be followed by another high measurement. This form of variability can be stipulated in the model specification by using the autoregressive covariance structure. An autoregressive structure assumes that observations which are taken close together are more similar than those taken further apart. The other covariance structure that is used in this thesis is the unstructured form. The unstructured covariance structure makes no assumptions about the relationship between observations and uses the data to estimate their relationship[520].

3.2.3 Model building strategies

Each analysis chapter of this thesis includes a statistical methods section which outlines the statistical methods used to analyse the data. Explanatory variables will be introduced along with any transformations necessary to normalise the data. Most of the explanatory variables analysed in this thesis are well known in HIV and HCV research, however, detailed explanations will be given where new or novel approaches have been used.

A mixture of baseline variables and time-updating variables are used where appropriate. Baseline variables, such as baseline age, sex and height, which by definition are fixed over time, are used to determine the long term association with an outcome of interest. Time-updating variables make use of data that are collected at regular intervals, such as HIV viral load and CD4 cell count which are measured at 3-monthly intervals for many individuals in EuroSIDA, which vary over time and can reflect disease progression or pathogenesis. Time-updating variables determine the short term association with an outcome of interest.

When using statistical models, univariable analysis is performed followed by multivariable analysis including all those variables statistically significant at the 10% level (p-value <0.1). In some cases variables that are not significant in univariable analysis will still be included in multivariable analysis. This is appropriate when clinical knowledge suggests that these variables are responsible for variability in the outcome of interest. In some analyses with

few endpoints it may have been decided *a priori* using empirical knowledge which variables it would be most interesting to analyse.

Sensitivity analysis has also been used frequently to check the sensitivity of the model to specific variables. Further details are given in each subsequent chapter in the methodology and discussion sections.

3.2.3.1 Handling missing data

Where data are incomplete or missing various methods of missing data handling have been incorporated. When few data are missing and omitting cases with missing values does not drastically reduce the analysis population, the complete-case method has been used. This method has been showed to be unbiased when the missing data is not missing as a function of either the outcome of interest or the model error term[521]. However, when many data are missing the complete-case method is highly inefficient as it leads to a large number of cases being excluded.

When fairly large amounts of data are missing indicator variables have been used to create missing categories that can be included in statistical modelling. However, this approach is known to produce biased estimates in some circumstances[521]. Therefore, in instances where further investigation of the effect of missing data is required multiple imputations have been used to impute the missing data. Multiple imputations fill in missing data by estimating what the data are likely to be multiple times using the characteristics of individuals with complete data. These multiple imputations are then combined so that variability associated with the multiple imputations is included in the standard error of the overall parameter estimates, which has been shown to provide unbiased estimates[522].

Comparing the results from a multiple imputations analysis with the main analysis using indicator variables then allows for description of what effect the missing data are having on the statistical inference.

3.2.4 Summary

The statistical models and methodology presented in this chapter provide an illustration of the statistical methods employed throughout this thesis. Each analysis that follows will use specific methodology tailored to the research question of interest. Although care has been taken to minimise the effect of inherent bias, each analysis will have its own strengths and weaknesses. A more detailed explanation of the methods employed for each analysis is included in each of the following five chapters, while the strengths and weaknesses of each piece of work are presented in the discussion as well as in Chapter 9 of this thesis.

Chapter 4

The natural history of HCV RNA levels during chronic HCV infection among HIV/HCV coinfected individuals

4.1 Introduction

As discussed in Chapter 2 Section 2.2, coinfection with HCV is one of the most clinically important comorbidities in the HIV-positive population. Of the 35 million people currently living with HIV worldwide, approximately 20% have chronic HCV infection[383;523;524]. In recent years mortality rates attributable to HIV infection have decreased as a result of antiretroviral therapy[525]. As a result, end-stage liver disease has assumed increasing importance as a cause of death among the coinfected population[404;526;527]. This is especially true for people who acquired HIV via injecting drug use (IDU), in whom HCV is common as a result of shared transmission routes[383;524;526].

Anti-HCV antibody (HCVAb) positivity has been associated with a higher rate of any-cause death and liver-related death among the HIV-positive population[24;404;528-530], while evidence presented in Section 2.2.6 suggests that plasma HCV RNA, along with HCV genotype and interleukin IL28B gene variant, has been shown to be one of the most important predictors of sustained virological response (SVR) to treatment with pegylated-interferon (peg-IFN) and ribavirin (RBV) in coinfected individuals[436;462;523;526;531]. Furthermore, it has been reported that an HCV RNA measurement taken early after HIV seroconversion can predict progression to AIDS and death in individuals with high HCV viral loads[532].

4.1.1 HCV and cART

The use of combination antiretroviral therapy (cART) in coinfected individuals is complicated by an increased risk of antiretroviral-associated hepatotoxicity[533]. Coinfection with HCV has been consistently identified as the most significant risk factor for the development of liver enzyme elevations after initiation of cART[380]. One study reported that HCV coinfected individuals had a 2.5-fold greater risk of developing severe hepatotoxicity compared to HCV uninfected individuals[534]. Similarly, a study from the United States reported that HCV coinfection was independently associated with a greater than 2-fold increased risk of severe hepatotoxicity[535]. While these studies indicate that HCV coinfection increases the risk of elevations in liver enzymes after cART initiation, most studies indicate

that coinfected individuals treated with cART do not develop severe hepatotoxicity, with one study showing that 88% of coinfected people treated with cART did not develop hepatotoxicity, defined as a greater than 5-fold increase in alanine transaminase (ALT) or aspartate transaminase (AST)[536]. Accordingly, most HIV/HCV coinfected individuals can be treated safely with cART, though they should be monitored closely for the development of signs and symptoms of hepatotoxicity[537].

There is also some evidence that use of cART can partially restore CD4 T-cell responses to core HCV peptides, meaning that successful responses to cART among the HIV/HCV coinfected population can lead to increased immune responses to HCV. Further, an increased immune response to HCV may lead to long term reductions in HCV RNA levels and potential clearance of HCV[389;538;539]. Indeed the Swiss HIV Cohort Study group recently showed that, after successful treatment with cART, HIV/HCV coinfected individuals had an increased response to HCV core peptides and had a slight decrease in HCV RNA levels compared to pre-ART levels[540].

Despite the risk of hepatotoxicity, it is strongly suggested that successful responses to cART can restore cellular immune responses to HCV antigens and lessen the progression of chronic liver disease while improving the response to anti-HCV therapy. Therefore, the early initiation of cART is supported among HIV/HCV coinfected individuals[376;541]. However, cART does not appear to fully correct the adverse effect of HIV infection on HCV related outcomes and should not distract from the main aim of HCV therapy, which is HCV eradication with appropriate treatment[389;542].

4.1.2 HCV RNA and clinical outcomes

HCV viral loads and their relationship to liver disease and other clinical outcomes have been the subject of many studies of HCV monoinfection with conflicting results. A study by *De Moliner et al* found no correlation between histological outcome and HCV RNA levels concluding that 'the extent of replicative activity of HCV does not seem to play a role in the modulation of associated hepatic disease'[543]. However, other studies have reported that viral titre may well influence the severity of liver damage. *Mita et al* found that HCV RNA titres were significantly higher in individuals with chronic active HCV, with the highest viral loads seen among those with cirrhosis and hepatocellular carcinoma[544], while *Naito et al* describe inflammatory changes in the portal tracts with the severity depending on the replicative levels of HCV[545]. However, all of these studies were cross-sectional and relatively small, including less than 100 participants.

Two larger studies by *Gretch et al*[546] and *Adinolfi et al*[547], which included 121 and 298 participants respectively, also found that high HCV RNA titres correlate with the most severe forms of liver damage. *Adinolfi* in particular states that 'liver damage is correlated with HCV RNA levels and that high HCV viral load acts together with steatosis in accelerating the progression of liver injury'[547]. The contradictions in the findings of these studies may be explained by the timing of the HCV RNA and liver damage determinations. A major limitation to these studies is that many of them compare the extent of liver injury at a given point in time to a single estimate of viral load, while none of them consider the predictive ability of HCV RNA or the role of coinfection with HIV.

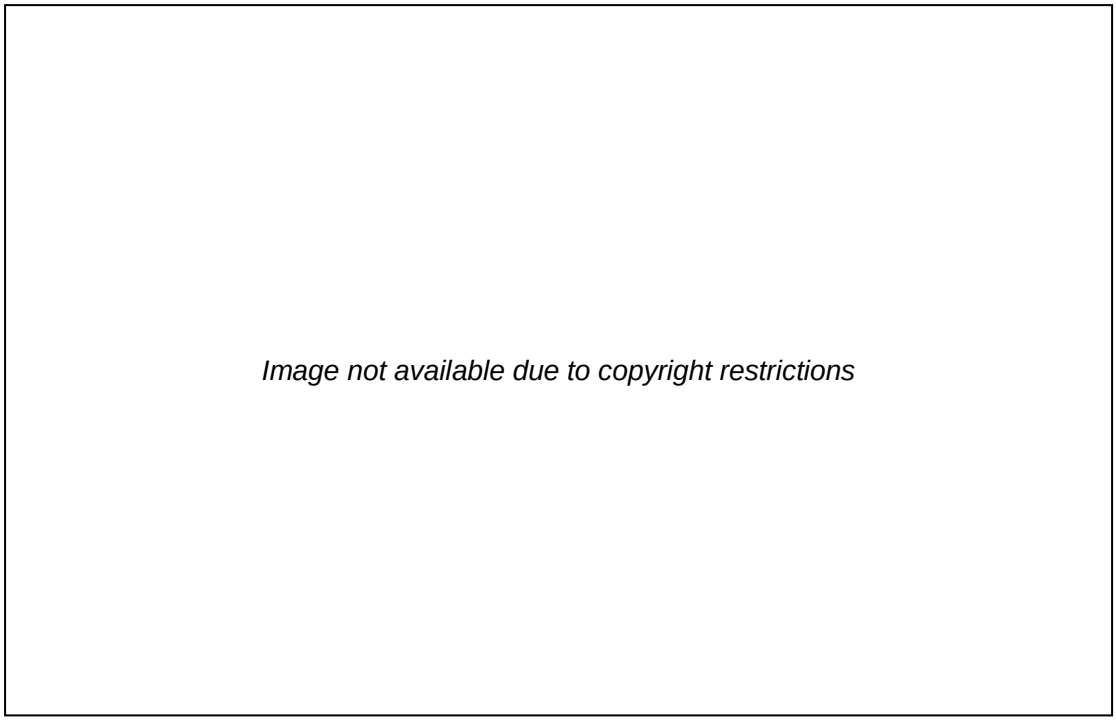
More recently HCV RNA levels and their association with liver-related death among coinfected individuals was the subject of a large EuroSIDA study with a long duration of follow-up. *Rockstroh et al,* in a study including 13,025 HIV-positive individuals, 30% of whom were HCVAb positive at baseline, over a median duration of follow-up of 7 years, found that HCVAb positive individuals had 9-fold increased risk of liver-related death, while among HCVAb positive individuals those with viremic infection were at 2-fold increased risk compared to those with aviremic infection[548]. However, when going on to examine the role of HCV RNA in more detail, using time updated HCV RNA measurements, there was no evidence of a dose response relationship between HCV RNA and liver-related death (Figure 4.1)[548].

Although HCV is primarily a disease of the liver it has been shown to have a detrimental effect on other organ systems. Recent studies of HIV/HCV coinfected individuals have shown an association between HCV viremia and progression to chronic kidney disease (CKD). *Mocroft et al*, in 3,441 participants taking antiretroviral therapy from a clinical trial of HIV-positive individuals, reported that those with high levels of HCV RNA (>800,000 IU/ml) had 3-fold higher odds of developing CKD compared to HCVAb negative participants[549]. Further, the authors found some evidence to suggest that the odds of developing CKD increased as HCV RNA levels increased. A similar study from the EuroSIDA cohort by *Peters et al* also found that participants with high HCV RNA (>500,000 IU/ml) were at the highest risk of developing CKD, suggesting a contribution from active HCV infection towards the pathogenesis of CKD[26].

4.1.5 Natural history of HCV RNA

The natural history of HCV RNA levels and how they vary over time is the subject of recent research. A study of 60 chronically HCV monoinfected individuals by *Yeo et al*[550], including 445 HCV RNA measurements with a median of 8 measured per person, analysed changes

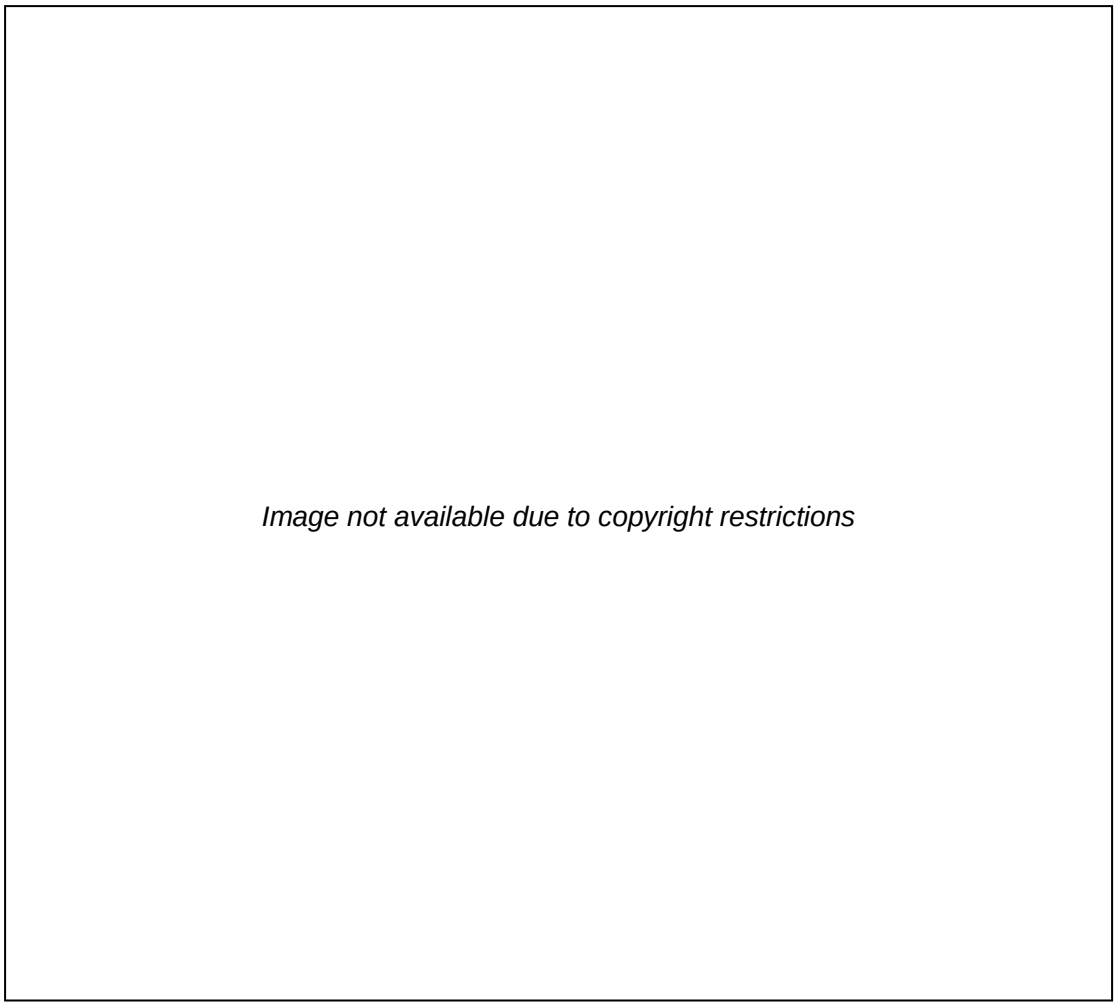
Figure 4.1 Progression to clinical events according to HCV RNA titre[548]



over time in HCV RNA levels. The authors found that HCV RNA levels varied over time by less than one log in 62% of individuals and less than 1.5 logs in 84%, while also noting no apparent association between HCV RNA titre and disease severity, as measured by raised transaminases (Figure 4.2). However, although this study included a relatively high number of measurements per person, the findings are limited by the short duration of the study, which was conducted over a median of 40 months.

In a study by *Thomas et al* that included 969 IDU HCV infected individuals, 48% of whom were coinfected with HIV, changes over time in HCV RNA were analysed using consecutive pairs of HCV RNA measurements[551]. In total 901 pairs of measurements were analysed, with a median of 5.7 (interquartile range (IQR): 5.5 - 6.0) months between measurements, and the median change in the magnitude of HCV RNA from visit-to-visit was 0.26 $\log_{10}$ copies/ml[551]. The authors found that reported IDU in the interval between HCV RNA measurements was associated with higher visit to visit increases in HCV RNA than those not reporting IDU. HIV infection, along with other sociodemographic factors, was not found to influence changes over time in HCV RNA levels. During the relatively short observation period in this study, less than 2 years, nearly equal proportions of individuals experienced increases or decreases in HCV RNA levels. However, duration of HCV infection was found to be a significant predictor of higher HCV RNA levels, indicating that serum HCV RNA may increase over time[551].

Figure 4.2 Stability of HCV-RNA and its lack of correlation with disease severity in asymptomatic chronic hepatitis C virus carriers[550]



A small study of 17 HIV/HCV coinfected patients by *Eyster et al* found that after an initial increase in HCV RNA following HIV seroconversion, HCV RNA levels remained stable during the 2 to 5 years following HIV seroconversion, however, after 5 to 13 years post seroconversion, HCV RNA had increased markedly[384]. In a more recent prospective study of 264 coinfected IDUs, 54% HIV-positive, with a median of 3 HCV RNA measurements per individual each separated by a median interval of 1 year, *Fishbein et al* report that HCV RNA levels did increase significantly over time in HCV monoinfected individuals, albeit a small increase of 0.025 $\log_{10}$ per year, but remained stable in HIV-positive individuals. The authors also note that HCV RNA levels were strongly positively associated with HIV RNA levels and identified HIV coinfection and HCV genotype 1 as predictors of higher HCV RNA levels[526].

4.2 Aims

The main aim of this chapter was to examine the natural history of HIV/HCV coinfection with regards to changes over time in HCV RNA levels prior to treatment for HCV. This study can add to the body of work on this topic by including a large number of individuals prospectively followed over a long period time. Of particular interest is to investigate which factors affect baseline HCV RNA levels and which factors affect changes over time in HCV viral load. It is also important to consider the role of treatment with cART with regards to its effect on HCV RNA levels as this is of potential interest to the clinical management of these individuals.

A further aim of this study was to discover if any factors of interest were predictive of HCV RNA levels reaching a clinically significant threshold. HCV viral loads >800,000 IU/ml have been associated with a poor response to traditional HCV treatment with pegylated-interferon and ribavirin, and progression to CKD in coinfected individuals.

4.3 Methods

4.3.1 Patient selection

The D35 update of the EuroSIDA database included 16,594 HIV-positive individuals from 105 centres across Europe, Israel and Argentina. Figure 4.3 shows the breakdown of how HIV/HCV coinfected individuals were selected for inclusion in this study. There were 14,324 individuals with known HCVAb status of whom 4,664 were antibody positive. From those 2,670 had data available on HCV RNA levels and of those 2,115 were positive (79.2%). From the 2,115 individuals positive for HCV RNA there were 1541 individuals with data recorded in international units (IU)/ml.

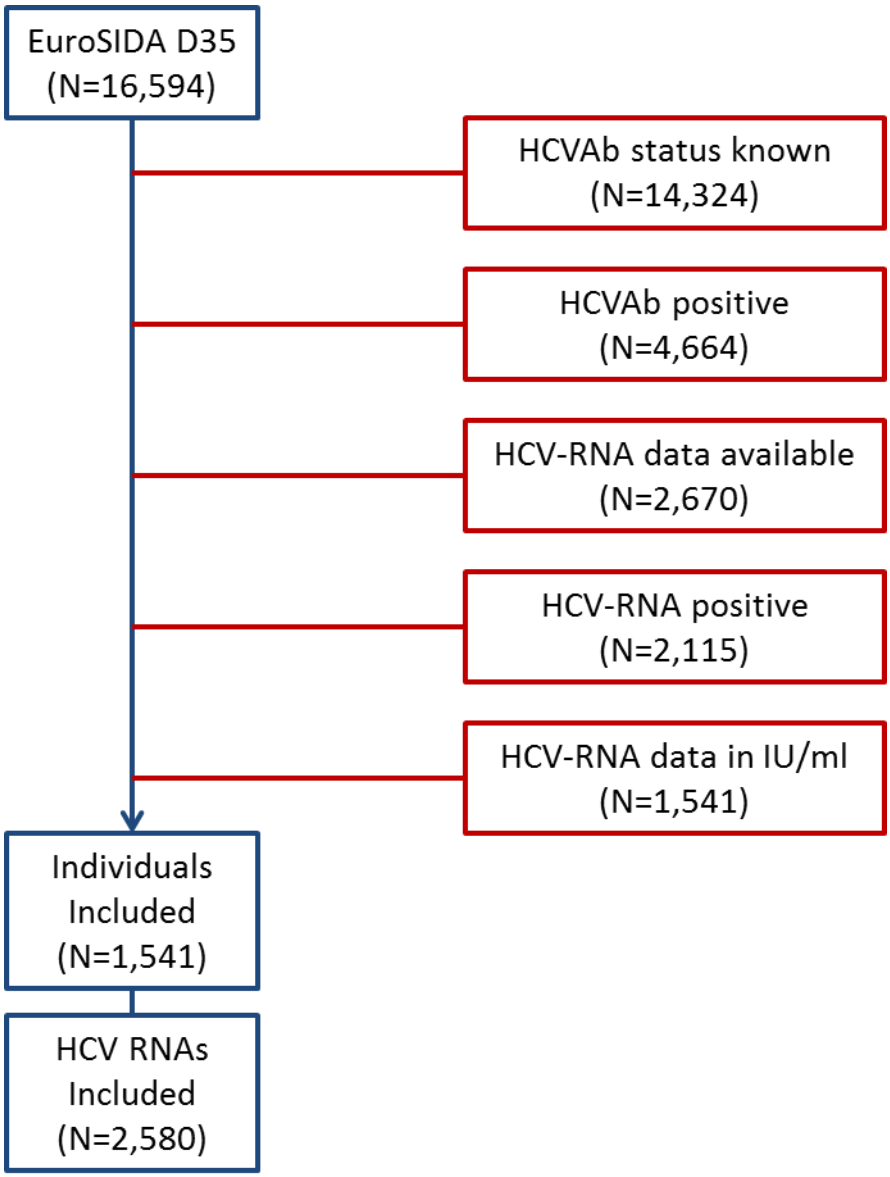
EuroSIDA HCV RNA data is recorded in either IU/ml, which has now become the universal standard of measurement, or copies/ml from historical data. In order for data that are recorded in copies/ml to be converted to IU/ml information about the assay used to determine the HCV RNA titre is required. Unfortunately, this information is not available in EuroSIDA for data in copies/ml. Efforts have been made to find an applicable conversion factor for these data, however, the nature of this study which specifically looks at changes over time in HCV RNA means that it is important to only include data collected in IU/ml. This way I can be sure that any observed changes between serial HCV RNA measurements are genuine and not due to measurement error associated with conversion from copies/ml to IU/ml.

HCV RNA measurements that were below the level of detection, which is 615 IU/ml, were taken as 615 IU/ml. The follow-up of those who clear the virus and remain undetectable was censored at the date of their first undetectable test. Measurements that were above the upper limit of detection, which varied between centres and was as low as 8,000 IU/ml from some historical data, were excluded from this study as participants that had multiple measurements above the range of detection would appear to have a perfectly flat HCV RNA level over time, which is highly unlikely to be the case. However, this was a minor occurrence with just 41 measurements from 39 individuals having to be excluded. Further, this did not alter the number of individuals included in the analysis shown in Figure 4.3.

4.3.2 Statistical methods

For the purpose of this study baseline was defined as the first positive HCV RNA measurement during prospective follow-up. Individuals were followed from baseline until their last HCV RNA measurement while HCV treatment naïve and HCV RNA positive. There were 399 participants that started HCV therapy and had their follow-up right censored at the date of initiating HCV treatment.

Figure 4.3 Analysis population inclusion criteria



Linear mixed models were used to assess which factors were associated with HCV RNA levels measured on the $\log_{10}$ scale. Such models allow for investigation into whether HCV RNA levels vary at baseline and whether HCV RNA levels change over time by accounting for within subject variability in the covariance structure. An unstructured covariance structure was used which allows the model to estimate the covariance between sequential HCV RNA measurements within subjects. The explanatory variables considered were:

- Time from first HCV RNA measurement
- Age/Sex/Race
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)

- HIV transmission risk group
- HCV genotype
- Time HIV antibody positive
- Calendar year of baseline HCV RNA measurement
- HBsAg status (time updated)
- HIV viral load (time updated)
- CD4 cell count (time updated)
- cART status (time updated)

cART status is a definition of whether a patient is receiving certain elements of an HIV treatment regimen. A PI regimen is defined as containing a PI and at least two other ARVs not including an NNRTI. An NNRTI regimen is defined as containing an NNRTI and at least two other ARVs not including a PI, and a more inclusive definition of any cART was defined as at least three ARVs from any drug class. Time from baseline HCV RNA measurement and the intercept were included as random effects in the model, all other variables were included as fixed effects.

To test the linearity of HCV RNA changes over time 2 indicator variables were tested which allowed the rate of change in HCV RNA over time to vary at 3 months and 12 months after baseline. Further, interactions between time and the covariates included in the mixed model were tested to see whether the rate of change in HCV RNA levels over time differed according to the levels of the covariates.

Secondary analyses in this chapter focused on assessing which factors were associated with rapid increases and decreases in HCV RNA over time, and with reaching a clinically significant threshold of 800,000 IU/ml. Rapid increases and decreases over time were defined *a priori* as those greater than the 90^{th} percentile among the individuals with more than 1 HCV RNA measurement in the analysis based on individual-level linear regression. Separate multivariable logistic regression models were used to describe factors associated with the odds of rapid increases or decreases in HCV RNA adjusted for the factors listed above. A similar multivariable logistic regression model was used to describe factors associated with having an HCV viral load ≥800,000IU/ml at any time during the course of follow-up, adjusted for the factors listed above.

4.4 Results

4.4.1 Generalizability and baseline characteristics

During the patient selection process for this chapter 1,994/4,664 HCVAb positive individuals were excluded as they did not have HCV RNA data available. To consider the generalizability of the results in this chapter multivariable logistic regression, adjusting for the covariates listed above, was used to assess how the excluded individuals differed from those that were included in the analysis. Individuals without HCV RNA data available were older at the time of their HCVAb positive test result (odds ratio (OR): 1.37 (95% confidence interval (CI) 1.20 – 1.56; *P*<0.0001), per 10 years older) and were more likely to reside in Eastern Central or Eastern Europe (OR: 3.31 (95% CI 2.29 – 4.79; *P*<0.0001) and OR: 8.05 (95% CI 5.45 – 11.88; *P*<0.0001) compared with Western Europe, respectively) than those with HCV RNA data.

Baseline characteristics of the 1,541 individuals that were included in the analysis are shown in Table 4.1, stratified by whether taking cART at baseline, defined as 3 ARVs from any class. Included individuals were predominantly white (91%), male (69%), IDUs (73%), and from Southern (35%) and Western Central Europe (23%); the HCV genotype distribution was G1 (58%), G2 (3%), G3 (36%) and G4 (14%). Eighty one percent were HBsAg negative, with 6% HBsAg positive and 13% with unknown HBsAg status.

There were significant differences between the populations on and off cART at baseline. Those that were off cART were younger (33.9 vs. 38.4 years; *P*<0.0001), while a higher proportion of individuals on cART were from Southern Europe and a lower proportion from Eastern Europe (37.4% vs. 28.2% and 4.9% vs. 22.7%; *P*<0.0001). Though HIV transmission risk groups and HCV genotypes were generally well matched between those on and off cART; there were a higher proportion of MSM that were on cART (10.5% vs. 5.3%; *P*=0.02) and a higher proportion with HCV genotype G3 among those off cART (31.8% vs. 23.7%; *P*=0.0003). Median CD4 cell count was well matched between those on and off cART, however, HCV viral loads were higher among those on cART (5.85 vs. 5.78 IU/ml; *P*=0.0019) and HIV viral loads were more often below the level of detection (60.1% vs. 11.8%; *P*<0.0001) as one might expect of cART-treated individuals.

It is important to point out that while on the $\log_{10}$ scale a difference in HCV RNA of 5.78 vs. 5.85 IU/ml looks minimal it actually equates to a rather large difference in absolute numbers. The $\log_{10}$ scale is exponential and a change from 5 to 6 equates to a change from 100,000 to 1,000,000 in absolute numbers. Therefore, the difference between an HCV viral load of 5.78 IU/ml and 5.85 IU/ml corresponds to a change of 105,386 IU/ml on the

Table 4.1 Baseline characteristics

N (%)	*Total (N=1541)*	*Off cART* (N=393)*	*On cART* (N=1148)*	*P-value[1]*
Gender				
Male	1070 (69.4)	263 (66.9)	807 (70.3)	0.21
Female	471 (30.6)	130 (33.1)	341 (29.7)	
Race				
White	1405 (91.2)	365 (92.9)	1040 (90.6)	0.17
Non White	136 (8.8)	28 (7.1)	108 (9.4)	
Age (Years (Median (IQR)))	37.5 (32.1 – 42.1)	33.9 (27.6 – 39.0)	38.4 (33.6 – 43.0)	<0.0001
Region of Europe				
South	540 (35.0)	111 (28.2)	429 (37.4)	<0.0001
West Central	348 (22.6)	78 (19.9)	270 (23.5)	
North	229 (14.9)	69 (17.6)	160 (13.9)	
East Central	246 (16.0)	42 (10.7)	204 (17.8)	
East	145 (9.4)	89 (22.7)	56 (4.9)	
Argentina	33 (2.1)	4 (1.0)	29 (2.5)	
HIV Exposure Group				
MSM	142 (9.2)	21 (5.3)	121 (10.5)	0.020
IDU	1117 (72.5)	303 (77.1)	814 (70.9	
Haemophiliac	49 (3.2)	9 (2.3)	40 (3.5)	
Heterosexual	186 (12.1)	49 (12.5)	137 (11.9)	
Other	47 (3.1)	11 (2.8)	36 (3.1)	
HCV Genotype				
1	896 (58.1)	227 (57.8)	669 (58.3)	0.0003
2	39 (2.5)	4 (1.0)	35 (3.1)	
3	397 (25.7)	125 (31.8)	272 (23.7)	
4	209 (13.6)	37 (9.4)	172 (15.0)	
Baseline HBsAg Status				
Positive	95 (6.2)	31 (7.9)	64 (5.6)	0.012
Negative	1241 (80.5)	325 (82.7)	916 (79.8)	
Unknown	205 (13.3)	37 (9.4)	168 (14.6)	

Baseline CD4 Cell Count				
Cells/mm^3 (Median (IQR))	347 (200 – 518)	356 (204 – 510)	340 (197 – 526)	0.12
Baseline Log10 HCV RNA				
IU/ml (Median (IQR))	5.82 (5.31 – 6.25)	5.78 (5.15 -6.13)	5.85 (5.37 – 6.30)	0.0019
HIV RNA <400 copies/ml	702 (47.6)	45 (11.8)	657 (60.1)	<0.0001

IQR - Inter quartile range; MSM – Men who have sex with men; IDU – Injecting drug user; HCV – Hepatitis C virus; HBsAg – Hepatitis B surface antigen; cART – Combination antiretroviral therapy

Baseline in this study was defined as the date of the first available HCV RNA measurement

***cART defined as at least 3 antiretrovirals from any class**

[1]P-value from Chi-square test for comparison of proportions or Kruskal-Wallis test for comparison of population medians

Baseline CD4 cell count and HIV viral load are taken as the closest measurement prior to baseline, up to 6 months prior, if no measurement is available in this window then a value up to 3 months after baseline is used

unlogged scale, or a 17.5% increase. To ensure that the magnitude of change in HCV RNA is properly conveyed, results in this chapter will refer to changes on the $\log_{10}$ scale and the percentage change in absolute numbers. This simply entails back-transforming the estimates from the model so that they become multiplicative, therefore, both estimates are from the same model.

At baseline the median time from the first documented HIV antibody positive test result among the included individuals was 9.2 years (inter quartile range (IQR): 4.8 – 12.9), while the median follow-up time, i.e. the time from the first till the last HCV RNA measurement, was 5.0 years (IQR: 2.8 – 8.3). There were 575 individuals that had at least two HCV RNA measurements, while the median number of measurements per person was 2 (IQR: 1 – 3, range: 1 – 10). The median time between consecutive HCV RNA measurements within individuals was 1.7 years (IQR: 0.7 – 4.1). There were 2.580 HCV RNA measurements included in total.

4.4.2 Exploratory data analysis

4.4.2.1 Correlation between HCV RNA and liver transaminases

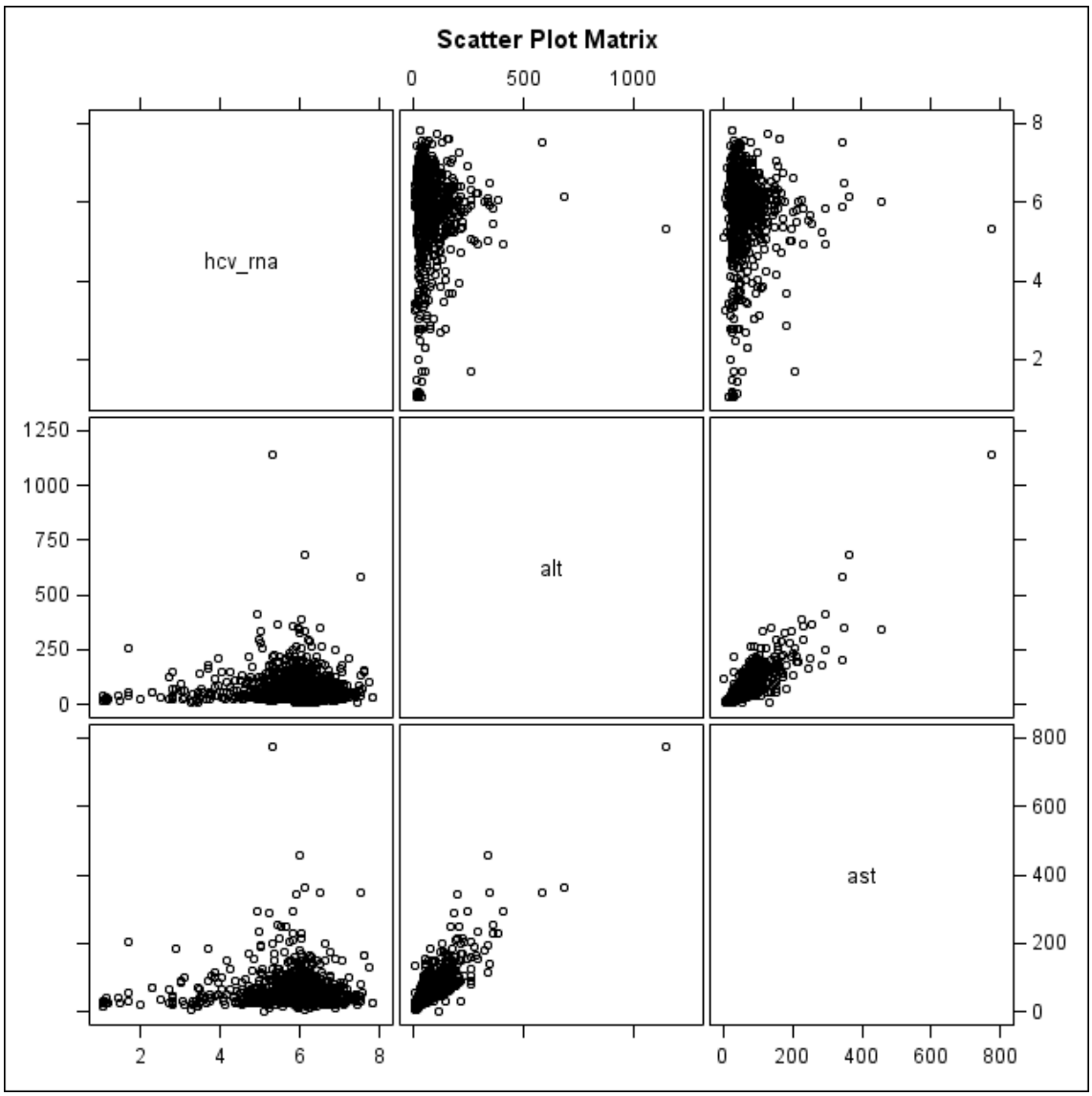
Of the 2,580 HCV RNA measurements included in this analysis, there were 1,144 from 765 individuals that had alanine transaminase (ALT) and aspartate transaminase (AST) measured within 3 months of the HCV RNA measurement. These data were pooled and used to describe correlations between HCV RNA, ALT and AST.

Figure 4.4 displays scatter plots showing the correlation between HCV RNA on the $\log_{10}$ scale, and ALT and AST in IU/ml. Whereas there was strong agreement between ALT and AST measurements and a clearly identifiable positive correlation (Pearson's correlation coefficient (R)=0.82; P<0.0001), as one would expect, neither ALT (R=0.08; P=0.0083) or AST (R=0.05; P=0.071) displayed any meaningful correlation with HCV RNA levels. The correlation between HCV RNA and ALT may have been statistically significant and the correlation between AST and HCV RNA borderline statistically significant, however, this was most likely a consequence of the relatively large sample size and the power of the statistical test rather than the identification of a meaningful association.

4.4.2.2 Individual HCV RNA profiles

Figure 4.5 displays 20 randomly selected individual HCV RNA profiles from those with at least three HCV RNA measurements, stratified by whether they were taking cART at baseline or not. Clearly there appears to be a large amount of natural variation in HCV RNA

Figure 4.4 Scatter plots showing correlation between HCV RNA, ALT and AST



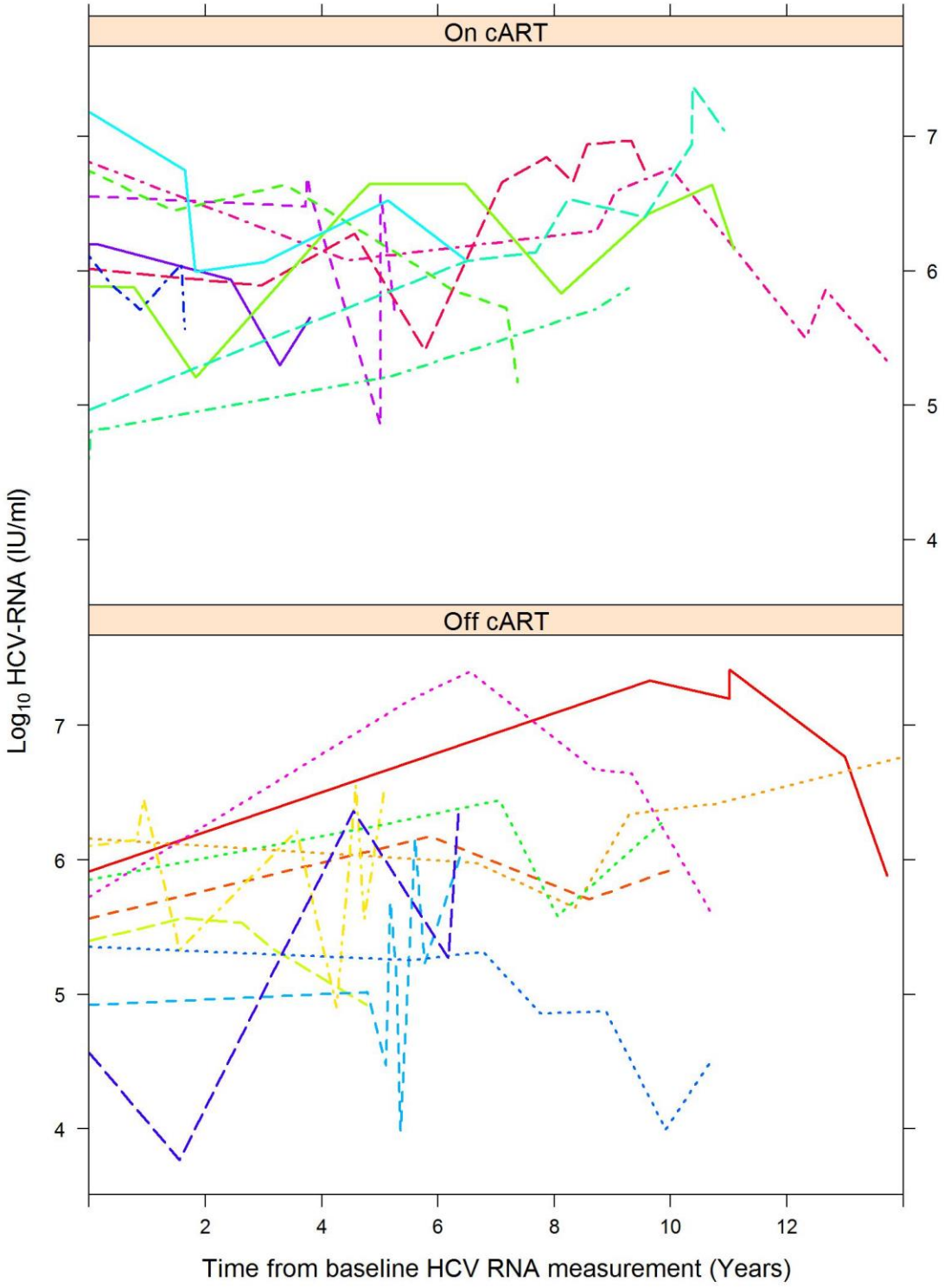
HCV RNA measured on the log_{10} scale; ALT and AST measured in IU/ml

levels. Though this is a small subset of the whole population there appears to be less variation among patients that were on cART at baseline compared to those that were not, with more drastic increases and decreases in HCV viral load seen among those not on cART at baseline.

4.4.3 HCV RNA levels at baseline and changes over time

The least squares mean estimates of HCV RNA levels within each stratum of covariates included in the mixed model, along with percentage differences within stratum and changes over time in HCV RNA, are shown in Table 4.2. Least squares mean estimates estimate the mean HCV viral load in each strata controlling for confounding by other factors. All

Figure 4.5 Twenty randomly selected individual HCV RNA profiles split by baseline use of cART



1,541 individuals in this study population contribute to estimates of baseline levels of HCV RNA, while the 575 individuals with at least two HCV RNA measurements contribute to the estimates for the change over time in HCV RNA.

4.4.3.1 Baseline HCV RNA

Baseline HCV RNA levels were lower for HCV genotypes 2 to 4 compared with genotype 1 (percent difference -25.5% (95% CI -39.1% to -8.8%; *P*=0.0044)). There was also some evidence to suggest that baseline HCV RNA levels were lower among those in the haemophiliac HIV transmission group compared with the IDU transmission group (percent difference -42.9% (95% CI -68.5% - 3.6%; *P*=0.065)) although the number included in the haemophiliac risk group was low and this only reached borderline statistical significance. Examples of these baseline differences are shown in graphical form in Figures 4.6 and 4.7. As the differences are at baseline only, these differences represent parallel lines, the small increase over time common to each stratum is associated with taking any form of cART as discussed below.

There was borderline to weak statistical evidence to suggest that HIV RNA levels above 400 copies/ml were associated with higher HCV RNA levels (percent differences 28.0% (95% CI -1.4% - 66.3%; *P*=0.064), 32.4% (95 CI -0.8% - 76.7%; *P*=0.057) and 32.5% (95% CI -6.5% - 87.6%; *P*=0.11) for HIV RNA 400-1,000, 1,000-10,000 and >10,000 copies/ml, respectively, compared with <400 copies/ml). Refitting the model including HIV RNA as a binary variable (<400 copies/ml vs. ≥400 copies/ml), unsuppressed HIV RNA was a strong predictor of HCV RNA, with HCV RNA levels 30.4% higher when HIV RNA was above 400 copies/ml (95% CI 4.9% – 62.0%; *P*=0.017), compared to HIV RNA levels <400 copies/ml. Further, when fitting HIV RNA as a continuous variable on the $\log_{10}$ scale a 1 log change in HIV RNA was associated with an 10.9% increase in HCV RNA (95% CI 2.3% – 20.2%; *P*=0.012).

Table 4.2 Least squares estimates of HCV RNA levels and changes through time

		Difference within strata			*Change in HCV RNA over time*		
	LS means at baseline* (Log_{10} IU/ml)	***%***	***95% C.I.***	***p-value[1]***	***% per year***	***95% C.I.***	***p-value[†]***
cART status							
None	5.50	0	-	-	27.6	(6.1, 53.5)	0.0098
All cART	5.61	26.8	(-3.0, 65.8)	0.083	2.6	(-1.1, 6.5)	0.17
PI regimen	5.59	9.4	(-13.4, 38.4)	0.45	3.4	(-0.2, 7.2)	0.068
NN regimen	5.65	27.4	(-24.0, 113.5)	0.35	2.0	(-5.2, 9.7)	0.59
HCV Genotype							
1	5.62	0	-	-			
2, 3, 4	5.49	-25.5	(-39.1, -8.8)	0.0044			
Baseline Age							
< 30	5.48	0	-	-			
30 – 40	5.56	20.9	(-12.8, 67.5)	0.25			
40 – 50	5.58	27.3	(-12.3, 84.8)	0.20			
> 50	5.60	30.8	(-21.0, 116.5)	0.30			
Gender							
Male	5.58	0	-	-			
Female	5.53	-11.0	(-28.9, 11.4)	0.31			
Race							
White	5.53	0	-	-			
Non White	5.58	10.6	(-24.9, 62.3)	0.61			
Region of Europe							
South	5.56	0	-	-			
West Central	5.60	10.7	(-17.1, 47.8)	0.49			
North	5.59	8.4	(-21.4, 49.7)	0.62			
East Central	5.63	19.7	(-15.0, 68.4)	0.31			
East	5.54	-2.6	(-39.5, 56.9)	0.92			
Argentina	5.41	-29.2	(-66.3, 48.6)	0.36			
HIV Transmission Group							

Injecting Drug User	5.65	0	-	-
MSM	5.52	-25.3	(-48.1, 7.4)	0.12
Haemophiliac	5.41	-42.9	(-68.5. 3.6)	0.065
Heterosexual	5.57	-17.2	(-40.0, 14.3)	0.25
Other	5.63	-3.5	(-45.1, 69.5)	0.90
HIV RNA (copies/ml)				
< 400	5.47	0	-	-
400 – 1,000	5.58	28.0	(-1.4, 66.3)	0.064
1,000 – 10,000	5.59	32.4	(-0.8, 76.7)	0.057
> 10,000	5.59	32.5	(-6.5, 87.6)	0.11
CD4 Cell Count (cells/mm^3)				
> 500	5.54	0	-	-
350 – 500	5.55	2.2	(-22.6, 35.0)	0.88
200 – 350	5.54	-1.1	(-23.0, 27.2)	0.93
< 200	5.59	11.7	(-11.7, 41.4)	0.36
HBsAg Status				
Negative	5.61	0	-	-
Positive	5.50	-21.9	(-47.9, 16.9)	0.23
Unknown	5.55	-13.6	(-36.9, 18.4)	0.36

***Least squares mean estimates of HCV RNA levels at baseline in each stratum averaging other confounding factors**

[1]P-value for comparisons of absolute HCV RNA levels within stratum

[†]P-value for increase through time of HCV RNA

Figure 4.6 Differences in baseline HCV RNA levels by HCV genotype for individuals taking cART

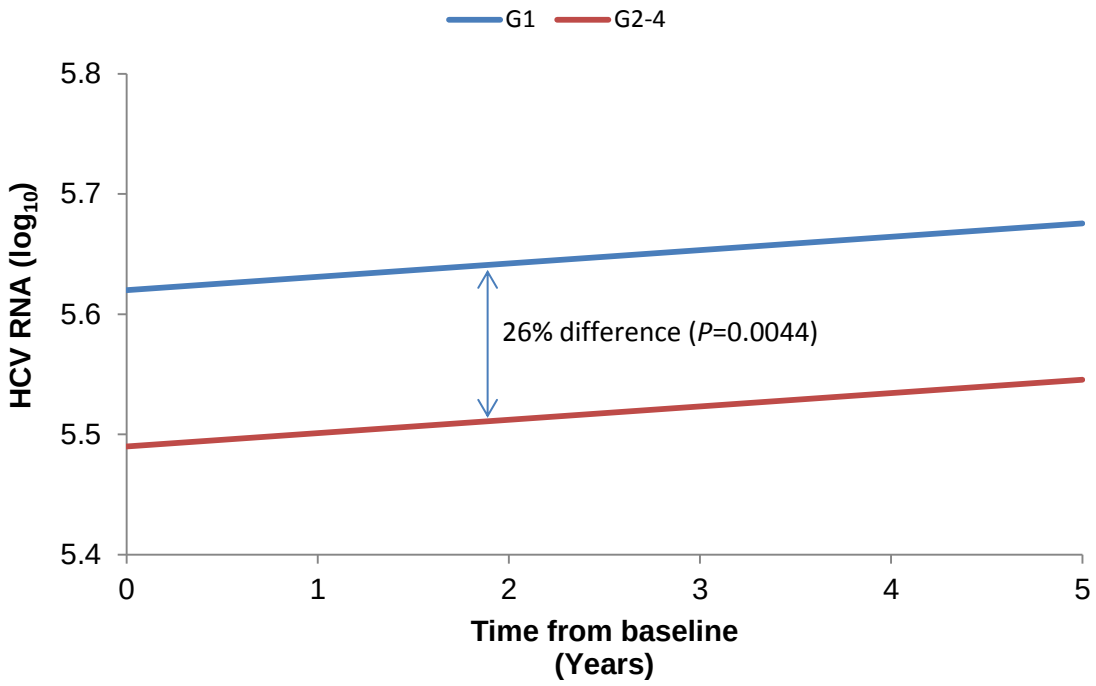
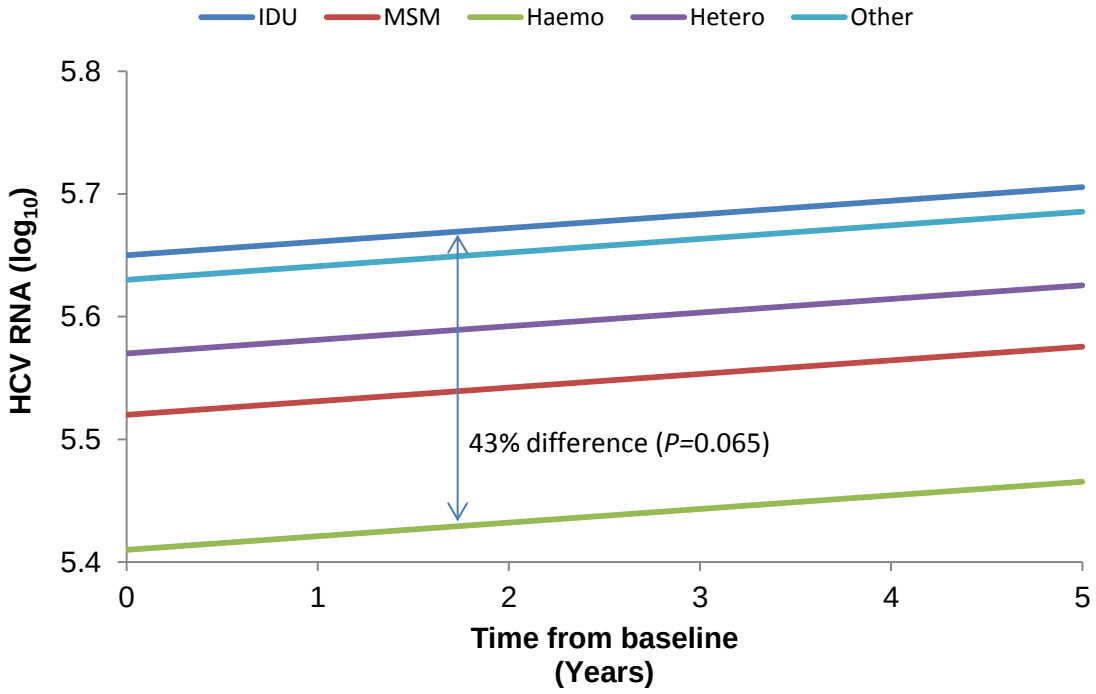


Figure 4.7 Differences in baseline HCV RNA levels by HIV transmission route for individuals taking cART



IDU: injecting drug user, MSM: men who have sex with men, Haemo: haemophiliac, Hetero: heterosexual

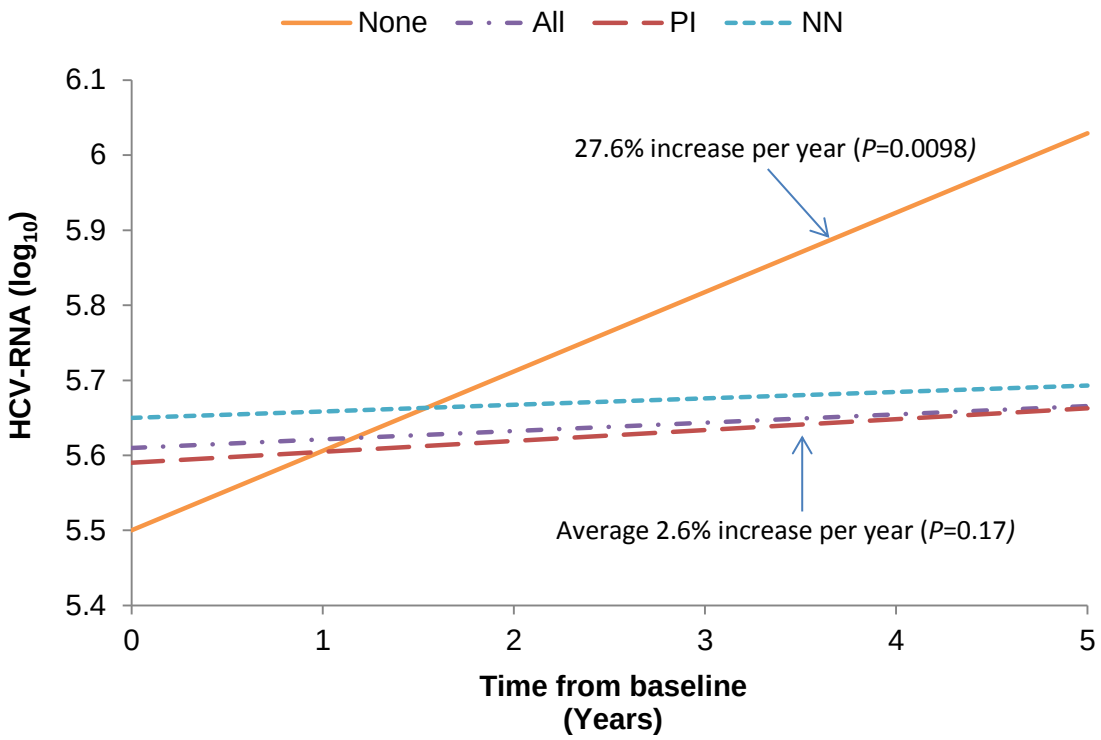
When fitting age per 10 years and CD4 cell count on the $\log_2$ scale as continuous variables neither approached statistical significance (*P*=0.31 and *P*=0.81, respectively).

4.4.3.2 Changes over time in HCV RNA

Among individuals not taking cART, HCV RNA levels increased by a mean of 27.6% per year (95% CI 6.1% – 53.5%; *P*=0.0098). Among individuals taking any form of cART, HCV RNA levels remained stable over time, with an estimated non-significant increase of 2.6% per year (95% CI -1.1% - 6.5%; *P*=0.17). When separating the different cART regimens HCV RNA levels also remained stable through time among those taking PI-containing and NNRTI-containing regimens (non-significant increases of 3.4% per year (95% CI -0.2% - 7.2%; *P*=0.068) and 2.0% per year (95% CI -5.2% - 9.7%; *P*=0.59), respectively). Figure 4.8 shows the estimated HCV RNA trajectories for each cART category.

Two indicator variables that allowed the change over time in HCV RNA to vary 3 months and 12 months after baseline were tested in the model, however, neither approached statistical significance (both *P*>0.4). Further, a quadratic time variable was tested and did not approach statistical significance (*P*>0.5). Therefore, there was no evidence to suggest that the change in HCV RNA over time was non-linear.

Figure 4.8 Estimated HCV RNA trajectories by cART regimen



Estimated HCV RNA trajectories match the baseline HCV RNA levels and change through time estimated from the mixed model.

Apart from the interaction between cART use and time (*P*=0.023), interactions between the other covariates and time in the mixed model did not reach statistical significance (all *P*>0.15). This finding indicates that the rate of change in HCV RNA over time was affected by use of cART in this population but not by the other covariates. For example, although the results in Table 4.2 show that baseline HCV RNA is significantly higher for HCV genotype 1 versus HCV genotypes 2, 3 and 4, there is no evidence to suggest that the rate of change in HCV RNA after baseline differs by HCV genotype, as shown by the parallel lines in Figure 4.6. The effect of the significant interaction between cART use and time is displayed in Figure 4.8 where individuals that are not taking cART clearly appear to have a greater increase in HCV RNA through time compared to those taking cART.

4.4.3 Rapid changes in HCV RNA and reaching a clinically important threshold

Among the 575 individuals with multiple HCV RNA measurements, rapid increases and decreases in HCV RNA through time were defined using the 90th percentiles of the study population. For increases, this equated to a 10% increase per year, while for decreases this equated to a 7% decrease per year. In multivariable logistic regression adjusting for the covariates listed above those that had rapidly increasing HCV RNA were more likely to have HCV genotype 1 than 2, 3 or 4 (adjusted odds ratio (aOR) 1.37 (95% CI 1.01 – 1.86; *P*=0.042)), all other covariates were non-significant predictors of rapid HCV RNA increases (*P*>0.15). In a separate model, individuals with rapidly decreasing HCV RNA were less likely to reside in Eastern Central or Eastern Europe compared with Southern Europe (aOR 0.43 (95% CI 0.22 – 0.84; *P*=0.013) and aOR 0.40 (95% CI 0.17 – 0.94; *P*=0.035), respectively), all other covariates were non-significant predictors of rapid HCV RNA decreases (*P*>0.15).

A clinically important threshold for HCV RNA levels was discussed in the introduction to this thesis and briefly at the beginning of this chapter, with 800,000 IU/ml considered to be a clinically important titre. In multivariable logistic regression including the whole study population adjusting for the covariates listed above, HCV genotype 1 was a significant predictor of having an HCV viral load ≥800,000 IU/ml (aOR 1.45 (95% CI 1.17 – 1.77; *P*=0.0006) compared with genotypes 2, 3 and 4), along with residing in Western Europe (aOR 1.71 (95% CI 1.24 – 2.34; *P*=0.0010) compared with Southern Europe). All other covariates were non-significant predictors of reaching an HCV viral load of 800,000 IU/ml.

4.4.4 Sensitivity analyses

A number of sensitivity analyses were performed in order to further examine the increase in HCV RNA levels over time and to test the robustness of this finding (summarised in Table

4.3). First the patient population was restricted to those with ≥3 HCV RNA measurements available. The generalizability of the subset of individuals with ≥3 HCV RNA measurements available was assessed in logistic regression. In multivariable logistic regression adjusted for the covariates listed above, those with ≥3 HCV RNA measurements were less likely to have HCV genotype 1 (aOR: 0.83 (95% CI 0.69 – 1.0; *P*=0.049) compared with HCV genotypes 2-4), were younger (aOR: 0.75 (95% CI 0.65 – 0.86; *P*<0.0001) per 10 years), were more likely to reside in Western Europe (aOR: 4.0 (95% CI 3.1 – 5.1; *P*<0.0001)) and less likely to reside in Eastern Europe (aOR: 0.2 (95% CI 0.1 – 0.5; *P*<0.0001) compared with Southern Europe) and were more likely to be taking cART (aOR: 1.6 (95% CI 1.2 – 2.1; *P*=0.0043)), compared with those with <3 HCV RNA measurements.

Similar to the results in the main analysis, although with wider confidence intervals reflecting the decreased statistical power, the increase in HCV RNA levels through time among those not on cART was 38.9% per year (95% CI 0.7% – 91.8%; *P*=0.046). For those taking any form of cART HCV RNA was stable through time, with an estimated non-significant increase of 1.2% per year (95% CI -4.7% - 7.4%; *P*=0.70). For those taking a PI-based cART regimen, HCV RNA was again stable, with an estimated non-significant increase of 3.2% per year (95% CI -3.2% - 10.1%; *P*=0.33), while for those taking an NNRTI-base cART regimen HCV RNA was also stable, with an estimated non-significant increase of 0.2% per year (95% CI -33.2% - 56.3%; *P*=0.92).

A further sensitivity analysis focused on ensuring that poor quality of HCV RNA samples was not having an effect on the results presented in this chapter. Performing the main analysis of this chapter again in those with at least three HCV RNA measurements, this time using only data that were collected from clinical sites and not using data from stored samples. By doing so it is possible to exclude the possibility that changes in HCV RNA over time could be explained by degradation in stored plasma samples. In this sub-population of 169 individuals, HCV RNA levels increased 20.9% per year (95% CI -16.9% - 75.9%; *P*=0.32) among individuals not taking cART, although without reaching statistical significance as a consequence of the reduced power of this analysis. In those taking any cART HCV RNA levels were again remained stable over time, with an estimated non-significant decrease of 3.1% per year (95% CI -11.1% to 5.6%; *P*=0.47).

Table 4.3 Summary of cART-related effects examined in sensitivity analyses

Analysis	***Criteria***	***N included***	***% change in HCV RNA over time (95% CI)***
Main	All EuroSIDA HIV/HCV coinfected individuals with HCV RNA data available in IU/ml	1,541	No cART: 27.6% (6.1 – 53.5; *P*=0.0098) Any cART: 2.6% (-1.1 – 6.5; *P*=0.17) PI cART: 3.4% (-0.2 – 7.2; *P*=0.068) NN cART: 2.0% (-5.2 – 9.7; *P*=0.59)
Multiple HCV RNA measurements	Only those with ≥3 HCV RNA measurements	258	No cART: 38.9% (0.7 – 91.8; *P*=0.046) Any cART: 1.2% (-4.7% - 7.4%; *P*=0.70) PI cART: 3.2% (-3.2% - 10.1%; *P*=0.33) NN cART: 0.2% (-33.2% - 56.3%; *P*=0.92)
Multiple HCV RNA measurements reported from clinical sites only	Only those with ≥3 HCV RNA measurements reported from clinical sites	169	No cART: 20.9% (-16.9% - 75.9%; *P*=0.32) Any cART: 3.1% (-11.1% - 5.6%; *P*=0.47)

PI cART: PI-containing cART regimen, NN cART: NNRTI-containing cART regimen

4.5 Discussion

The natural history of HCV RNA

The analysis in this chapter examined the natural history of plasma HCV RNA in chronically infected HIV/HCV coinfected individuals. The main findings of this analysis show that among individuals not taking cART, HCV RNA levels increased significantly by 27.6% per year. This is in comparison to a non-significant 2.6% increase per year among coinfected individuals being treated with any form of cART. Furthermore, similar stability of HCV RNA levels was demonstrated when further classifying cART into PI- and NNRTI-based regimens, with small non-significant increases in the order of 3.4% and 2.0% per year, respectively.

These findings are in agreement with other work on the subject. *Fishbein et al*[526], as mentioned in the introduction to this chapter, reported small increases in HCV RNA in the region of 2% per year for coinfected individuals and 6% per year for HCV monoinfected individuals. The *Fishbein* study included only coinfected IDUs but remains comparable to this study as the majority of those included here were also IDU (73%). Unfortunately, the *Fishbein* study does not mention whether the individuals they included were treated with cART, however, the similarities between the two sets of results and the low level of HIV RNA reported suggest that they were.

In 2000 *Thomas et al* reported from a study of haemophiliacs in the UK that HCV RNA levels were stable in the long run[551]. However, the authors also reported on increasing HCV RNA levels during the first four years after HIV seroconversion[551]. One of the main benefits of the analysis presented in this chapter is that follow-up was included over a long period of time. The median time from the first HIV antibody positive test result to the first HCV RNA measurement was 9.2 years (IQR 4.8 – 12.9). Therefore, this analysis presents contrasting evidence to suggest that the increase in HCV RNA continues beyond the four year period after HIV seroconversion in the absence of treatment for HIV infection, assuming that the individuals included here were infected with HCV prior to or at the same time they became infected with HIV. For IDUs this is considered reasonable because of the shared transmission routes of HIV and HCV, as mentioned in the introduction to this thesis. This is considered less likely for MSM and heterosexuals, as sexual transmission of HCV is less efficient than that of HIV[313], however, as the majority of individuals included in this study were IDU, invalidity of this assumption is not a major concern.

The reported increase in HCV RNA levels among those not taking cART of 27.6% per year appears to be a striking finding, although due to the limited power the 95% CI is quite wide. However, this analysis has identified an association between treatment with cART and stable HCV RNA levels in coinfected individuals. It is unclear how much influence increasing HCV RNA levels will have on clinician choice when deciding whether to treat individuals for HCV while the association between HCV viral load, disease severity and liver disease remains unclear[550;552]. More weight will often be given to other factors, such as the level of fibrosis, HCV genotype, IL-28B gene variant and an individual's readiness for treatment[523;553]. However, as pointed out in the introduction to this chapter, with recent studies suggesting a link between high HCV viral loads (>500,000IU/ml and >800,000IU/ml) and progression to chronic kidney disease[26;549], increasing HCV RNA could potentially become more clinically important in the future.

HCV viral loads were seen to be higher among those with detectible HIV RNA. The least squares mean estimate for HCV RNA levels among individuals with detectible HIV RNA was 5.59 IU/ml compared to 5.47 IU/ml for those with HIV RNA below 400 copies/ml. This equates to 32% lower levels of HCV RNA among those with controlled HIV infection. Further, when fitting HIV viral load on a $\log_{10}$ continuous scale it was found to have a significant relationship with HCV RNA. A $\log_{10}$ increase in HIV RNA was associated with an 11% increase HCV RNA, which further supports the finding that treatment with cART can help to control HCV infection.

These findings, building on the evidence presented from the *Fishbein*[526] study, have potential implications for the treatment of individuals with HIV/HCV coinfection. Lower HIV viral loads predict lower HCV viral load, which is known to be one of the most important predictors of successful HCV treatment outcome with interferon-based therapy[554]. Therefore, it may be possible to improve the chances of SVR to HCV treatment by controlling HIV viral load and indirectly stabilising HCV RNA levels in the absence of HCV therapy. However, a short term transient increase in HCV RNA levels has been reported in coinfected individuals initiating cART[555;556]. Further, another study has reported that individuals with low CD4 cell counts (<350 cells/mm^3) at cART initiation experienced a continuous increase in HCV RNA levels for the 48 week duration of the study[557]. A recent study with a long follow-up period also found that there was a small increase in HCV RNA 6 months after cART initiation, but a significant decrease in HCV RNA at 70 months after cART initiation[540].

Other studies which have examined the course of HCV RNA levels at three and 12 months after the initiation of cART have produced conflicting results[555]. Different studies have found

increases and decreases in HCV RNA levels at these time points following cART initiation, although often the changes were not statistically significant and the results were based on a small number of individuals[558]. It is difficult to examine the potential non-linearity of the course of HCV RNA due to cART initiation in EuroSIDA at these time points as 75% of individuals in this study were already taking cART at baseline, with a median time on cART of 2.7 years (IQR 1.0 – 5.3). Adding interaction terms to the multivariable mixed model allowing the rate of change in HCV RNA to differ at 3 and 12 months after baseline were excluded from the final model as they were not statistically significant, along with a quadratic time variable. Therefore, the current analysis presents no evidence to suggest that HCV RNA changes over time are non-linear. However, the ability of this study to detect deviations from a linear change over time is limited by the low number of HCV RNA measurements available per participant.

Predictors of clinically significant levels of HCV RNA

HCV genotype 1 was a significant predictor of higher HCV RNA levels at baseline compared with genotypes 2, 3 and 4, although changes in HCV RNA over time did not differ significantly between genotypes. Similarly, previous studies have shown an association between higher HCV RNA levels and HCV genotype 1[524;526], although this is not true of all studies[550;552]. However, these studies have tended to include relatively few individuals, especially with genotypes other than G1. Increases in HCV RNA of more than 10% per year (the 90th percentile) were also found to be associated with HCV genotype 1, as was reaching a threshold of 800,000 IU/ml, which has been reported to be a predictor of a poor response to interferon-based treatment for HCV[436]. These findings highlight HCV genotype 1 as the most difficult for which to control HCV RNA levels. EuroSIDA has previously published a comparison of baseline HCV RNA levels by genotype[559], but this study adds important information by considering longitudinal changes in HCV RNA and the effect of cART use for genotypes 1, 2 , 3 and 4 in a large number of individuals.

This study found no evidence that HBsAg status influenced HCV RNA levels, baseline HCV RNA levels were 22% lower among HBsAg positive individuals compared to HBsAg negative individuals (5.50 IU/ml vs. 5.61 IU/ml), but the difference did not reach statistical significance. Many previous studies have reported that the presence of HBV may favour the clearance of HCV RNA in individuals with multiple chronic viral hepatitis infections[559-561], including a previous EuroSIDA study[559]. That this analysis did not find HBsAg status to be associated with HCV RNA levels could be explained by a number of reasons. Firstly, there are very few individuals included in the study that were HBsAg positive at baseline, just 6.2%, which would make it difficult to detect statistically significant differences. Conversely, it is possible that among the previously multiply coinfected individuals,

suppression of HCV had already occurred as a result of the presence of HBV some time ago, that these individuals were HCV RNA negative when testing began and therefore excluded from the analysis in this chapter.

When testing interactions between covariates and time, this study found no evidence to suggest that the rate of change in HCV RNA differed by any of the factors included in the mixed model, other than use of cART. However, the analysis had limited power to detect these differences in the rate of change because of the relatively low number of HCV RNA measurements per individual. Further studies are required to fully understand the rate of change in HCV RNA levels and how it may differ according to genotype or other factors, which could potentially be revisited in time when more data are collected.

With increasing interest in HCV coinfection in the HIV population EuroSIDA has committed to collecting as much HCV-related data as possible, including HCV RNA, with the aim of improving the quality and quantity of this data. Monitoring efforts have since been tailored to focus on sites with HIV/HCV coinfected individuals and monitors are instructed to collect all previously recorded HCVAb, HCV RNA and HCV genotype data that may have previously been missed during data collection. More recently, EuroSIDA has begun to recruit a new cohort of participants. In keeping with the HIV/HCV coinfection research agenda it was decided to recruit coinfected individuals only and data on HCV RNA, HCV genotype along with liver fibrosis and a range of fibrosis biomarkers will collected from entry into the study for the new cohort.

4.5.1 Limitations

The analysis presented in this chapter has several limitations, most importantly it must be noted that there were relatively few individuals included in EuroSIDA with multiple HCV RNA measurements. Of the 1,541 individuals included in this analysis, 575 had at least 2 HCV RNA measurements and contributed to the estimates of changes over time in HCV RNA. At least 2 measurements are required per individual in order to estimate not only the change over time in HCV RNA but interactions between time and the other covariates. The reduced number of individuals with at least 2 HCV RNA measurements will have had an effect on the model's ability to detect these interactions. Further, there were 258 individuals included with at least 3 HCV RNA measurements. At least 3 measurements are required per individual in order to estimate non-linear trends in HCV RNA. Therefore, the relatively few individuals with at least 3 measurements would have had an effect on the model's ability to describe non-linear trends in HCV RNA. Many more measurements per person would ideally be required to accurately describe these patterns. As such I have focused this analysis on long term changes in HCV RNA which assume a linear trend over time,

allowing for random variation around the mean. Although there are valuable conclusions to draw from the analysis, more data per person would have allowed me to perform more detailed analyses.

Another limitation of this analysis is that HCV RNA data were included from both stored samples and routine clinical care. While there is the possibility that stored samples could degrade over time during storage it is also possible that data from stored samples and clinical care are inherently different. Whereas collection of a stored sample does not necessarily reflect a change in the clinical condition of the patient, it is likely that a clinician would decide to perform an HCV RNA test as part of routine care as a response to a change in the condition of the patient. However, sensitivity analysis using only the data that were collected during the clinical setting resulted in similar conclusions to the main analysis of increasing HCV RNA in those not taking cART and stability among those that were, indicating that this bias is not a major concern.

Further limitations relate to which data are collected in EuroSIDA. The route of HIV transmission is collected but the route and date of HCV transmission are not. Therefore I have assumed the same route of transmission applies to both viruses. As the majority of people included in this analysis are IDU, it is probably reasonable to assume that they also acquired HCV via this medium due to the effectiveness of HCV to transmit via blood to blood contact, as was discussed in the introduction to this thesis. However, it is less likely to be the case in MSM due to the less frequent transmission of HCV during sexual contact.

4.5.2 Conclusion

The analysis in this chapter demonstrated that, while HCV RNA levels increased during long-term follow-up in HIV/HCV coinfected individuals not taking cART, among those who had initiated cART HCV RNA levels were stable over time. This and the fact that there was an association between HCV RNA and HIV RNA suggest that earlier treatment with cART could help to prevent increases in HCV RNA levels in HIV/HCV coinfected individuals. Further, HCV genotype 1 was associated with higher baseline HCV viral load, increased odds of having a rapid increase in HCV RNA and an HCV viral load ≥800,000 IU/ml.

Chapter 5

Temporal changes and regional differences in the uptake of treatment for HCV among HIV/HCV coinfected individuals in EuroSIDA

5.1 Introduction

The substantial declines in HIV-related mortality, as a consequence of the introduction of combination antiretroviral therapy (cART), have seen liver-related mortality assume increasing importance among HIV-positive individuals[562-564]. Progression of liver disease is common with hepatitis C virus (HCV) infection and known to be accelerated in the presence of HIV[413;565]. Treatment for HCV offers the possibility of eradicating HCV within a defined treatment period. This is potentially advantageous for the subsequent management of the individual with HIV and every coinfected individual should therefore be considered for treatment when the benefits of therapy outweigh the risks.

As discussed in Chapter 2 Section 2.2.6, development of new direct-acting antivirals (DAAs) for treatment of HCV has been a fast moving area of research over the past few years. As such, many second line DAAs with fewer side effects and excellent cure rates are now available, including interferon-free regimens, subject to cost[2]. However, the analysis presented in this chapter focuses on data collected over the period just before the release of second line DAAs, when interferon-based treatment was still the standard of care and first line DAAs boceprevir and telaprevir were the state of the art, known as triple therapy. Consequently, the introduction and discussion relating to treatment for HCV in this chapter refers to the period around the year 2011.

5.1.1 European treatment guidelines

A detailed description of the evolution of care for HCV is given in Section 2.2.6 of the introduction of this thesis. In short, treatment of HCV has traditionally revolved around the use of interferon (IFN), which is a natural cellular protein that can induce an antiviral state in target cells and recruit immune cells to help fight infection[367]. The first clinical trials of IFN for treatment of HCV monoinfection were published in the mid-1980s, with poor treatment response rates of approximately 10-15%[367]. However, refinement of IFN-based therapy meant that by the mid-1990s, approximately when follow-up in EuroSIDA began, HCV

positive individuals were treated with IFN plus ribavirin with cure rates in monoinfection approaching 40% and 30% in HIV/HCV coinfection[428].

The final refinement of IFN-based therapy was a switch to pegylated-IFN (peg-IFN) plus ribavirin. Peg-IFN is a slow release formulation of IFN which can be administered once a week. Clinical trials in the early 2000s showed that this new formulation cured approximately 50-60% of HCV monoinfected and up to 40% of HIV/HCV coinfected individuals[428-430]. By 2011, the first generation of direct-acting antivirals (DAAs), boceprevir and telaprevir, for treatment of HCV were coming to market. These new drugs introduced an era of triple therapy, peg-IFN plus ribavirin and a DAA, and saw cure rates approach 75% for easy-to-treat HCV genotype 1 infected individuals[437;441;442].

In 2011, European AIDS Clinical Society (EACS) guidelines recommended that all HIV/HCV coinfected individuals with significant liver fibrosis should be considered for HCV therapy given their increased risk of liver-related death[1]. Information on liver fibrosis staging is important for making therapeutic decisions in coinfected individuals; however, liver biopsy is no longer mandatory for considering treatment of chronic HCV[1], as discussed in Section 2.2.5.3. Interferon-based therapy was particularly recommended for individuals with a high likelihood of achieving sustained virological response (SVR), such as those with HCV genotypes 2 or 3, genotype 1 patients with an IL28B CC genotype or genotype 1 patients with a previous relapse under dual therapy which could be retreated with triple therapy (telaprevir or boceprevir, plus interferon and ribavirin)[1].

Where fibrosis staging was available, whether from liver biopsy or Fibroscan®[421], for results demonstrating lack of or minimal liver fibrosis (METAVIR F0-F1[420]), regardless of HCV genotype, treatment for HCV could be deferred[1]. While this was also the case for individuals with low chances of SVR with interferon-based treatments, for whom improved treatment options were expected to become available in the coming years[1]. Individuals with genotype 1 infection who could potentially be treated with DAA-based therapy but had expected adherence issues could also have had treatment deferred until easier to take, better-tolerated second line DAAs become available[1]. In such cases, fibrosis assessment should have been carried out periodically to monitor fibrosis progression[1].

When fibrosis staging showed significant liver fibrosis or worse (F2-F4) treatment was recommended but could have been deferred according to prior treatment history[1]. HCV treatment naïve individuals with easier to treat genotypes 2 and 3 were recommended to start dual therapy[1], while those with genotype 1 were recommended to start triple therapy, as shown in Figure 5.1[1;439]. The only exception being those who were non-responders to

previous therapy, in whom it may be beneficial to wait for future more efficacious treatment options[1].

HCV treatment should be preferentially offered to individuals with controlled HIV infection or those where HCV is detected early in the course of HIV infection[1]. In individuals where HCV infection is detected before the initiation of cART is necessary, treatment for HCV is advised[1]. For individuals with CD4 cell counts below 500cells/mm^3 but above 350cells/mm^3 early initiation of ART is recommended along with HCV treatment in order to maximise the potential for a successful treatment outcome[1;450;566]. However, in individuals with significant immunodeficiency categorised by CD4 cell counts below 350cells/mm^3, the CD4 cell count should be improved using cART prior to commencing anti-HCV treatment[1;450;566].

However, data from the APRICOT study of the efficacy of dual HCV therapy in HIV/HCV coinfected individuals[436] have shown that HCV therapy can be effective at lower CD4 cell counts. *Opravil et al*[567] showed that for individuals treated with optimum dual HCV therapy and well-controlled HIV infection, the rates of SVR were similar between those with CD4 cell counts below 200cells/mm^3 and those above 350cells/mm^3. With the exception of HCV genotype 1 where SVR rates were 13% in those with baseline CD4 below 200cells/mm^3 and 32% in those with baseline CD4 above 350cells/mm^3 (Figure 5.2)[567].

Figure 5.1 Management of HIV/HCV coinfected patients with HCV genotype 1 according to fibrosis stage and prior treatment outcome[439]

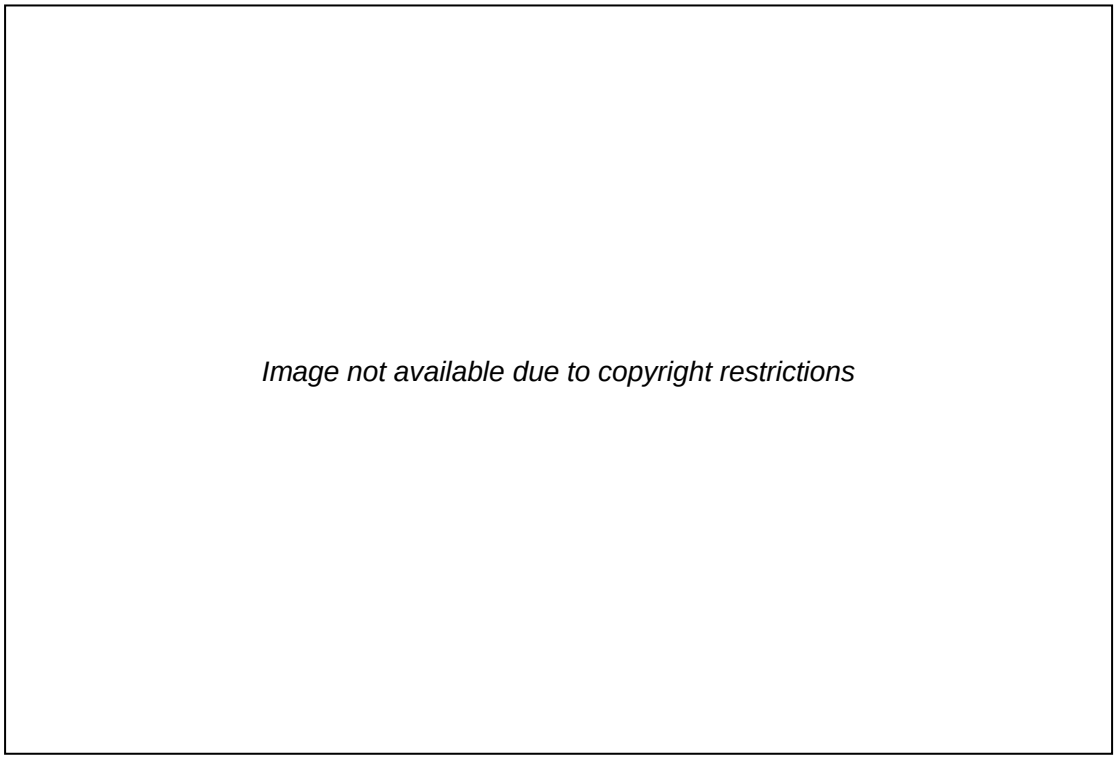


Figure 5.2 SVR rates according to baseline CD4 cell count strata for all patients (A), patients with genotype 1 (B), and patients with genotype 2/3 (C)[567]

EACS guidelines in 2011 also stated that individuals with a CD4 relative percentage greater than 25% were more likely to achieve SVR than those with a lower CD4 percentage[1]. The same study by *Opravil et al*[567] mentioned above also showed a clear trend of greater rates of SVR with higher levels baseline CD4 percentage. Specifically, for those taking optimal dual therapy the rates of SVR were 33%, 36%, 41% and 47% for those with baseline CD4 percentages of 2.5-19.1, 19.1-25.0, 25.0-32.1 and 32.1-69.3 respectively.

5.1.2 HCV drug pipeline – Direct-acting antivirals (DAAs)

Although the data analysed in this chapter include follow-up to 2011, when the gold standard of treatment for HCV infection was dual therapy with peg-IFN and ribavirin (RBV), it is important to consider which new drugs are on the horizon for the treatment of HCV as the potential availability and increased efficacy of these new drugs are factors that may have influenced clinicians to defer treatment over the study period.

As mentioned above and in more detail in Section 2.2.6, the approval of triple therapy for HCV with telaprevir or boceprevir and peg-IFN/RBV increased the cure rate among HIV/HCV coinfected individuals. However, triple therapy remains unsuitable for individuals either intolerant of or with contraindications to IFN or RBV[445]. Therefore, new therapeutic approaches offering improvements in efficacy, safety and tolerability are needed to address the currently unmet medical need[445]. Fortunately, research in the area of HCV treatment is fast-moving and it is hoped that new direct-acting antivirals (DAAs) will be able to fill the current gaps in available therapy[445].

More than 60 DAAs are now in clinical trials with a much greater number in preclinical development[445]. While data from early phase clinical trials are scarce for HIV/HCV coinfected individuals, there are a number of published studies of HCV-monoinfected individuals. The inclusion of telaprevir to peg-IFN/RBV has seen SVR rates increase from 45% to 75% and shortened the duration of treatment from 48 weeks to 24 weeks in over half of patients[440]. While the inclusion of boceprevir to peg-IFN/RBV has seen SVR rates increase from 40% to 68%[437], with important similarly sized improvements seen in treatment of previously treated responder-relapsers[441;442].

The most promising form of triple therapy in development currently includes TMC-435, which is another potent protease inhibitor[445]. In a large phase II clinical trial of treatment-naïve HCV genotype 1 individuals treated with TMC-435/peg-IFN/RBV there was documented SVR in over 90% of patients[568]. This combination also appears to be very well tolerated with no significant drug-related toxicity or direct drug interactions[568]. Further, similarly high SVR rates have been observed in phase II studies in HCV genotype 1

patients for triple therapy with the following experimental protease inhibitors; BI201335[569], MK7009[570;571], and the NS5A inhibitor BMS-790052[572].

Triple therapy incorporating a new DAA fails when HCV variants that are resistant to the protease inhibitor are not fully supressed by peg-IFN/RBV. In this case quadruple therapy has been trialled, including two different DAAs without cross-resistance and peg-IFN/RBV[445]. In a small phase II study of the DAA combination of protease inhibitor BMS-650032 and NS5A inhibitor BMS-790052 plus peg-IFN/RBV, virological breakthrough was prevented in all 10 individuals with 100% SVR[573]. However, while the newer triple and quadruple treatment combinations have better efficacy, safety and tolerability than the first wave of DAAs telaprevir and boceprevir, they are still not able to cater for individuals with contraindications to peg-IFN/RBV, with the real goal of HCV drug development targeting IFN-free regimens[445].

Development of a multiple DDA regimen for treatment of HCV is based on the lessons learned from HIV drug development. Different DAAs which target different steps of HCV viral replication are combined to attack the virus from multiple angles and increase viral suppression and reduce the emergence of resistance[445]. The first study of IFN-free combinations of DAAs in individuals with chronic HCV was the INFORM-1 study[574]. This proof of concept study, including 87 individuals with HCV genotype 1, demonstrated excellent viral suppression and prevented the emergence of resistance to either compound when treated with the nucleoside polymerase inhibitor meracitabine and the NS3/4A protease inhibitor danoprevir[574]. However, this was not a curative study and all patients rolled over onto peg-IFN/RBV after 14 days of DAA treatment.

More recently, promising results from the combinations of a number of DAAs have led to rapid progress in this area. The nucleoside polymerase inhibitor sofosbuvir (formerly known as GS-7977) has been combined with the NS5A inhibitor daclatasvir (formerly BMS-790052), with or without RBV with remarkable results[575;576]. Cure rates ranged from 88% to 100% after 12 or 24 weeks of treatment, regardless of prior treatment history, ribavirin use or HCV genotype[575;576]. Unfortunately, this combination looks to be left behind as Gilead (the owner of sofosbuvir) is looking to create a fixed dose combination with its own NS5A inhibitor ledipasvir.

The COSMOS phase II study of 167 individuals is pairing simeprevir (formerly TMC435) and sofosbuvir for 12 and 24 weeks, with and without RBV[577]. Again early results were outstanding, at post treatment week 8 (SVR-8) 96% of those receiving triple therapy and 92% of those receiving dual therapy without RBV were undetectable[577]. So far 24

individuals have been followed to post treatment week 12 (SVR-12) and all were undetectable[577].

The Gilead owned combination of sofosbuvir plus ledipasvir with or without RBV has also produced excellent results. In the ELECTRON trial, 100% of 25 treatment-naïve and 10 null-responder participants were cured after 12 weeks of treatment including RBV[578]. Sofosbuvir and ledipasvir are now available as a co-formulation in a single fixed-dose pill. In the LONESTAR phase II trial 100% of individuals treated with the fixed-dose combination for 12 weeks maintained undetectable HCV RNA 4 weeks after finishing treatment[579]. While 40/41 (97.6%) treated for 8 weeks remained undetectable 8 weeks after treatment[579]. The fixed-dose combination has since entered phase III trials.

Further, AbbVie's quad regimen containing ABT-450/r, a boosted HCV protease inhibitor co-formulated with ABT-267, an NS5A inhibitor, plus ABT-333, a non-nucleoside polymerase inhibitor, and RBV has resulted in almost universal cure rates among treatment-naïve and null-responder patients[580]. Bristol-Myers Squibb are also developing a three drug IFN/RBV-free in-house combination containing daclatasvir, a protease inhibitor (asunaprevir), and a non-nucleoside polymerase inhibitor (BMS-791325), with SVR rates almost 100% in early stage trials[581].

The above review of DAAs drug development reflects the state of drug development in 2011, when follow-up for this analysis ended. Consequently, some of the drugs mentioned are now approved for treatment of HIV/HCV coinfection in Europe and the United States. The full list of current state of the art treatments for HCV are discussed in Section 2.2.6.2.

5.1.3 Previous studies of HCV treatment uptake

Despite the recommendations detailed above, the extent to which HIV/HCV coinfected individuals are eligible for and start HCV therapy is not well documented in Europe, with previous studies from 2003-2006 showing a low proportion of individuals initiating therapy. In a small study by *Fleming et al* in 2003 the authors found that eligibility for treatment was low, out of 149 individuals with HIV/HCV coinfection just 30% were eligible for treatment, citing missed clinic visits, active psychiatric illness, active drug or alcohol use, decompensated liver disease, or medical illness as the main barriers to treatment[444].

A single cohort study from the Swiss HIV cohort by *Rauch et al* in 2005 also found low eligibility for HCV treatment among HIV/HCV coinfected individuals[443]. Among 107 chronically infected HIV/HCV individuals the authors found that 77% were not eligible for interferon-based treatment, with 73% of ineligible individuals having more than one

exclusion criteria and 33% having more than three[443]. The most frequent reasons given for exclusion were low CD4 cell counts, cytopenia, hepatic disorders other than hepatitis C, psychiatric illnesses, uncontrolled addiction, and poor adherence. Further, among the 25 individuals eligible for treatment 16 (64%) decided not to proceed, mainly due to fear of side effects[443].

A previous EuroSIDA study in 2006 by *Mocroft et al* documented increasing use of HCV treatment, however, its uptake remained scarce with variability across the regions of Europe[582]. By including 2,356 individuals enrolled in EuroSIDA and positive for HCVAb, the authors found that there was a 38% increase in the incidence of uptake of HCV treatment from before 1998 to 2004, but that in total just 7.6% of individuals had started interferon-based therapy[582]. However, as this study was only able to categorise HCV status using HCVAb testing and not HCV RNA, it is likely that the proportion of individuals eligible for treatment had been underestimated, as the denominator will have been inflated by individuals with aviremic HCV infection, who were HCVAb positive but did not require treatment as they had already cleared the virus.

In the largest European study to date including 6,433 HIV/HCV coinfected individuals, the *COHERE collaboration* in 2012 reported that 780 (12%) were treated for HCV[583]. However, this study also included a large proportion of individuals (71%) that were categorised as HCV positive on the sole basis of an HCVAb test, meaning that the proportion treated is likely to have been underestimated for the same reasons as the above previous EuroSIDA study. Further, although this study included a large number of individuals the median length of follow-up per individual was quite short at just 72 months (IQR: 39 - 108)[583].

Outside Europe the rates of treatment uptake also appear to be low. Three studies from the United States using the Veterans Affairs National Patient Care Database have documented modest increases in the proportion of eligible HIV/HCV coinfected individuals receiving interferon-based treatment. The first study in 2003 by *Fultz et al* found a high level of HCV treatment contraindications, including both medical and psychiatric comorbidities[584]. Of 65 coinfected individuals with indications for HCV therapy and free of contraindications for treatment, just 3% received interferon-based therapy[584].

The second study in 2006 by *Butt et al* included 6,502 coinfected individuals of which 12% were prescribed HCV treatment[585]. The authors state say that those of black race, Hispanic race, drug users, those with anaemia, bipolar disorder, major depression and mild depression were less likely to start treatment from fitting a multivariable logistic regression[585]. A more recently published study in 2012 by *Kramer et al* of 99,166 HCV

monoinfected individuals with detectable HCV viremia also found that 12% had received interferon-based treatment, with 55% of those going on to achieve SVR[586].

5.2 Aims

The aims of this chapter were to describe temporal changes and regional differences in the uptake of anti-HCV therapy among those with chronic HCV/HIV coinfection enrolled in the EuroSIDA cohort, and to identify factors associated with treatment uptake across Europe. One of the strengths of this analysis is the fact that EuroSIDA includes a large number of HIV/HCV coinfected individuals with well-characterised chronic HCV infection via HCVAb and HCV RNA testing. Further, this study includes a long period of follow-up, spanning 12 years, and can document how the incidence of treatment uptake has changed over time comparing the regions of EuroSIDA. It is also of particular interest to document the rate of completion of HCV treatment among those that receive therapy and factors that are associated with completing treatment.

A further aim of this study was to identify whether individuals with significant liver fibrosis (those with METAVIR stages ≥F2), in the most urgent need of treatment, have been selected for treatment. This is also an important feature of this analysis as no other European study of HCV treatment uptake has been able to categorise the importance of liver fibrosis when selecting individuals for treatment.

5.3 Methods

5.3.1 Patient selection

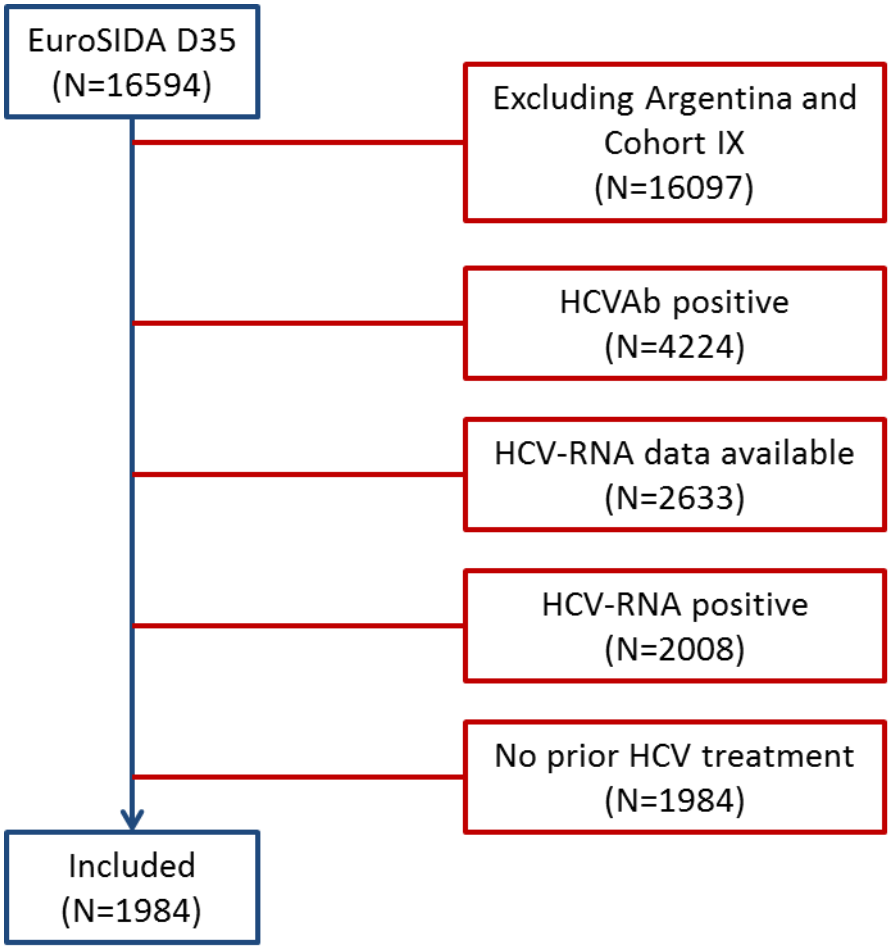
The D35 update of the EuroSIDA database included 16,594 individuals from 105 centres across Europe, Israel and Argentina. However, after consultation with Marcelo Losso, the EuroSIDA primary investigator for Argentina, it was decided that Argentina would not be included in these analyses as they were likely to include few individuals treated for HCV and the focus of the study is treatment uptake in Europe. In addition, as enrolment of EuroSIDA cohort IX had just initiated at the time of the D35 update, these individuals were excluded as they had no available follow-up. Figure 5.3 shows the breakdown of how individuals were selected for inclusion in this study. There were 16,097 individuals in EuroSIDA after excluding those from Argentina and cohort IX, of whom 4,224 had at least one HCVAb positive test. Of those 2,633 had data available on HCV RNA levels and 2,008 (76.2%) of these were positive. Finally, there were 1,984 coinfected individuals who had yet to receive treatment for HCV and were eligible for inclusion in this analysis.

Data on alanine transaminase (ALT), aspartate transaminase (AST) and platelet counts have been collected in individuals enrolled in the cohort since 1999 and 2005, respectively, and were used to calculate the AST to platelet ratio index (APRI[425]) as a marker for liver fibrosis. Data on liver biopsy and Fibroscan®[421] have been collected since 2010, with clinical sites requested to list all previous test results where liver biopsy was graded using the METAVIR scoring system[420], and return the histological report for internal validation. Plasma hyaluronic acid (HA) has been measured in all HCVAb positive individuals with stored plasma samples available, as has been described in Chapter 3 Section 3.1.2.2.

5.3.2 Statistical methods

As individuals included in this analysis were both HCVAb and HCV RNA positive, baseline was defined as the date of the first HCVAb/HCV RNA positive test result or recruitment to EuroSIDA, whichever occurred later. In analysis with the outcome of HCV treatment initiation, individuals are followed until their last visit, death or the date of starting HCV therapy. Throughout this analysis treatment for HCV is defined as treatment with at least interferon, although interferon-free regimens are today on the horizon they were not in 2011 when follow-up for this study ended. The gold standard for HCV treatment over the study period was peg-IFN/RBV[566], however, in earlier years interferon may have been given in its non-pegylated form. No distinction was made between the two possible formulations of interferon. Interferon may also have been previously prescribed without RBV, but IFN would have formed the backbone of all HCV treatment regimens over the study period[1].

Figure 5.3 Inclusions criteria; baseline is defined as the date of first HCVAb/HCV RNA positive test result or recruitment to EuroSIDA



Trends over time in starting HCV treatment were described using univariable Poisson regression models. Factors associated with HCV treatment uptake were investigated using univariable Poisson regression and those that were significant at the $P<0.1$ level were included in a multivariable model. The following explanatory variables were considered with time-updated variables noted in brackets:

- Age/Sex/Race
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- HIV transmission risk group
- AIDS diagnosis before baseline
- CD4 cell count (time-updated)
- HIV RNA (time-updated)
- HCV RNA (time-updated)
- HCV genotype

- HBsAg status (time-updated)
- cART status (time-updated, defined as currently receiving ≥3 ARVs of any class (yes/no))
- Calendar year
- ALT/AST levels (time-updated)

5.3.2.1 Fibrosis markers

Fibrosis levels among treated and untreated individuals have been summarised. A study by *Thein et al*[389] in 2008 found that the rate of progression of fibrosis staging is constant in HIV/HCV coinfected individuals, reporting annual transition probabilities of progressing to the next fibrosis grade of approximately 11%. Bearing this in mind, the last available fibrosis marker data was included up to two years prior to HCV treatment from individuals treated for HCV, along with the last available measurement in those that remain untreated. The proportions of treated and untreated individuals with significant fibrosis were compared using a combined definition of ≥F2 fibrosis from biopsy and Fibroscan®, HA > 100ng/ml and an AST to platelet ratio index (APRI) > 1.5, which have been shown to be accurate markers of significant fibrosis[587;588]. A Fibroscan® reading of >7.6kPa was used to identify ≥F2 fibrosis in accordance with a recent review of the subject[589].

5.3.2.2 Treatment completion

A full course of treatment with interferon was defined according to each individual's HCV genotype; see Chapter 2 Section 2.2.6. A full course is considered to have been completed after a minimum of 48 weeks of exposure to IFN for genotype 1 or 4 and a minimum of 24 weeks exposure for genotype 2 or 3[566]. However, because in the clinical setting treatment duration can occasionally be shorter than the standard 24/48 weeks, individuals who completed at least 80% of the expected minimum treatment duration were also considered to have completed a full course of therapy (i.e. 38.4 weeks for genotype 1 or 4 and 19.2 weeks for genotype 2 or 3), which is an established method also used in other studies of HCV treatment duration[586;590]. The median length of treatment duration by HCV genotype, along with the percentage of individuals completing a full course of treatment, were determined in individuals with known genotype and sufficient follow-up to have completed 80% of their treatment duration.

Predictors of completing a full course of HCV therapy were identified using a multivariable logistic regression model adjusted for the factors listed below:

- Age/Sex/Race
- HIV transmission risk group

- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- cART use at treatment initiation
- AIDS diagnosis before baseline
- HCV genotype
- CD4 cell count at treatment initiation
- HIV viral load at treatment initiation
- HCV viral load at treatment initiation
- Calendar year of treatment initiation

Where cART use at treatment initiation represents whether an individual was receiving cART (defined as ≥3 ARVs of any class) at the initiation of treatment for HCV. CD4 cell count, HIV viral load and HCV viral load at treatment initiation are the closest measurement prior to treatment initiation up to 6 months prior.

5.4 Results

5.4.1 Generalizability and baseline characteristics

The median follow-up time for the individuals included in this analysis was 168 months (IQR: 121 – 204 months). During the patient selection process for this analysis 1,591/4,224 HCVAb positive individuals were excluded as they did not have HCV RNA data available. In a multivariable logistic regression model adjusted for the factors listed above, HCVAb positive individuals without HCV RNA data available, had lower CD4 cell counts at baseline (adjusted odds ratio (aOR): 0.91 (95% C.I. 0.87 – 0.96; *P*=0.0002) per doubling), were more likely to reside in Eastern Europe (aOR: 6.45 (95% C.I. 4.63 – 8.99; *P*<0.0001) compared with Southern Europe), and were recruited to EuroSIDA in more recent years (aOR: 1.09 (95% C.I. 1.07 – 1.12; *P*<0.0001) per year later), compared with those positive for HCV RNA and included in the study.

Table 5.1 shows characteristics of the 1,984 coinfected individuals included in this analysis at baseline, and at the date of HCV treatment initiation or last follow-up for those not starting treatment. The populations of treated and untreated individuals were well matched on age at baseline, while a higher proportion of treated individuals were male (72.6% vs. 67.9%; *P*=0.048). A higher proportion of individuals that were treated for HCV resided in Southern Europe (41.1% vs. 33.0% for untreated individuals; global region *P*=0.0039) and belonged to the men who have sex with men (MSM) HIV transmission risk group (12.1% vs. 8.4%). A lower proportion of treated individuals belonged to the injecting drug use (IDU) HIV transmission risk group (68.9% vs. 74.5%; global transmission *P*=0.09). A higher proportion of individuals who went on to receive HCV treatment as opposed to remaining untreated were on cART at baseline (26.4% vs. 21.1%; *P*=0.014), consequently there was a higher baseline CD4 cell count in these individuals (median 290 cells/mm^3 (IQR 158.5 – 429) vs. 269 (145 – 400); *P*=0.017) and a higher proportion with undetectable HIV RNA (defined as <500copies/ml) (34.3% vs. 26.8%; *P*=0.0013).

At the time of HCV treatment or last follow-up, ALT levels were higher in treated individuals (data available in 409 vs. 1,308) (median 76 (IQR 49 – 120) vs. 44 (37 – 49) U/L for untreated individuals; *P*<0.0001), as were CD4 cell counts (median 479 cells/mm^3 (IQR 349 – 650) vs. 391 (227 – 614) for untreated individuals; *P*<0.0001). The proportion of individuals with HIV RNA <500 copies/ml was also higher among those treated for HCV (80.3% vs. 71.2%; *P*<0.0001).

Table 5.1 Patient characteristics at baseline and the date of last follow-up or HCV treatment initiation

%		*At Baseline*			*At Treatment or last follow-up*		
		Untreated (N=1483)	***Treated (N=501)***	***P-value****	***Untreated (N=1483)***	***Treated (N=501)***	***P-value****
Age	Med (IQR)	33 (28 - 38)	32 (28 - 37)	0.29	44 (37 - 49)	41 (35 - 46)	<0.0001
Male		67.9	72.6	0.048			
White		91.5	93.1	0.27			
Region of Europe	South	33	41.1	0.0039			
	West Central	21.6	18.1				
	North	17.5	12.9				
	East Central	16.2	17.7				
	East	11.7	10.3				
HIV transmission group	MSM	8.4	12.1	0.09			
	IDU	74.5	68.9				
	Heterosexual	10.4	11.7				
	Other	6.7	7.4				
HCV genotype	1	45.2	41.2	0.084			
	2	2.8	2.2				
	3	23.4	28.8				
	4	11.9	13.5				
	Unknown	16.7	14.5				
HBsAg status	Negative	60.8	65.1	0.21	85.0	88.7	0.12
	Positive	4.3	4.2		8.3	6.2	

	Unknown	34.9	30.8		6.7	5.2	
Started cART		21.1	26.4	0.014	86.7	86.3	0.85
ALT	N	123	38	0.44	1308	409	<0.0001
	Med (IQR)	51 (26 - 83)	49.5 (30 - 106)		44 (37 - 49)	76 (49 - 120)	
CD4	N	1482	501	0.017	1482	490	<0.0001
	Med (IQR)	268.5 (145 - 400)	290 (158.5 - 429)		391 (227 - 614)	479 (349 - 650)	
HIV RNA <500 copies/ml	% (95% CI)	26.8 (24.5 – 29.1)	34.3 (30.2 – 38.5)	0.0013	71.2 (68.8 – 73.5)	80.3 (76.7 – 83.9)	<0.0001
HCV RNA	N	1483	501	0.89	1470	430	0.64
	Med (IQR)	5.75 (5.17 - 6.23)	5.76 (5.21 - 6.21)		5.83 (5.18 - 6.30)	5.80 (5.36 - 6.29)	

ALT: alanine transaminase; cART: combination antiretroviral therapy; CI: confidence interval; HBsAg: hepatitis B surface antigen; IDU: injecting drug user; IQR: interquartile range; MSM: men who have sex with men.

ALT, CD4 cell count, HIV RNA and HCV RNA are the closest measurement prior to baseline, treatment or last follow-up up to 6 months prior.

***P*-values from Kruskal-Wallis test for difference in population distribution**

5.4.2 Temporal changes in the uptake of HCV treatment

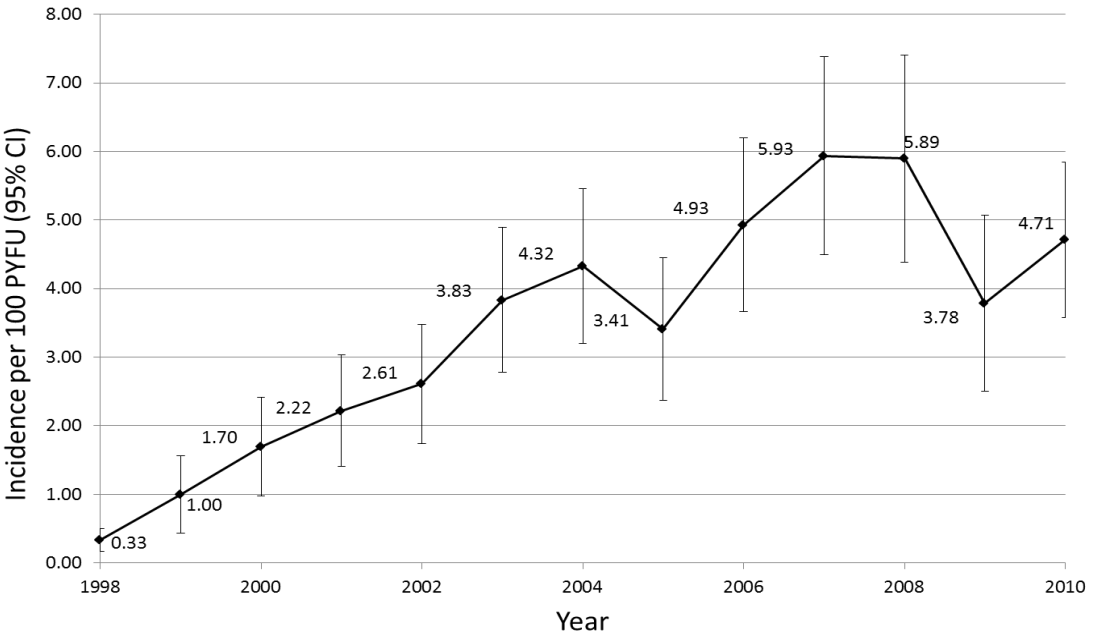
The median date of last follow-up for the study population was May 2011 (IQR: Sep 2005 – Nov 2011). By the end of follow-up, 501/1,984 (25.3%) coinfected individuals included in this study had received treatment for HCV with IFN/RBV or at least IFN, over a total of 18,303 person-years of follow-up (PYFU), giving an overall crude incidence of treatment initiation of 2.74 per 100 PYFU (95% C.I. 2.50 – 2.97).

Figure 5.4 displays how the incidence of treatment initiation has changed over time from 1998 to 2010. The overall incidence of HCV treatment in EuroSIDA increased from 0.33 (95% C.I. 0.16 – 0.50) per 100 PYFU in 1998 to 5.93 (4.49 – 7.38) in 2007 before it decreased to 3.78 (2.50 – 5.07) in 2009. Dividing the follow-up period into two sections, prior to the peak observed in 2007 and after 2007, univariable Poisson regression testing for temporal trends found that the incidence of treatment initiation increased by 27% per year between 1998 and 2007 (incidence rate ratio (IRR): 1.27 (95% C.I. 1.23-1.31; *P*<0.0001)), while in the period after 2007 there was a 12% decline per year in the incidence of treatment (IRR: 0.88 (0.79-0.98; *P*=0.020)).

Table 5.2 shows univariable and multivariable Poisson regression estimates for predictors of HCV treatment initiation. In the multivariable model, individuals who started treatment were more likely to reside in Southern Europe (adjusted incidence rate ratio (aIRR): 1.38 (95% C.I. 1.06-1.82; *P*=0.019) compared to Western Europe) and belong to the MSM HIV transmission risk group (aIRR: 1.36 (1.00-1.83; *P*=0.046) compared with IDU), compared with those not yet treated. Individuals that have been treated for HCV were also more likely to have current CD4 cell counts >350 cells/mm^3 (aIRR: 1.33 (1.06-1.67; *P*=0.013) compared to those with CD4 cell counts between 200 and 350 cells/mm^3) and were less likely to have CD4 cell counts <200cells/mm^3 (aIRR: 0.42 (0.27-0.65; *P*=0.0001) compared to people with CD4 cell counts between 200 and 350cells/mm^3), compared with untreated individuals.

Individuals treated for HCV were more likely to have current HIV RNA levels below 500 copies/ml (aIRR: 1.39 (95% C.I. 1.07-1.80; *P*=0.012) compared with HIV RNA >500 copies/ml), which along with the higher CD4 cell counts seen in treated individuals, indicates a better rate of well-controlled HIV infection through the use of cART compared with untreated individuals. Treated individuals were also more likely to have current HCV RNA >800,000 IU/ml (aIRR: 1.21 (1.00-1.47; *P*=0.049) compared with HCV RNA between 616 and 800,000 IU/ml) and raised ALT levels (upper normal range (uNR) < ALT < 3 times uNR, aIRR: 2.33 (1.83-2.96; *P*<0.0001); ALT > 3 times uNR, aIRR: 3.56 (2.61-4.86; *P*<0.0001) compared with ALT within the normal range), than untreated individuals.

Figure 5.4 Temporal changes in the uptake of treatment for HCV



1998 - 2007: (unadjusted IRR: 1.27 (95% CI: 1.23 -1.31; p<0.0001) per year)

2007 - 2010: (unadjusted IRR: 0.88 (95% CI: 0.79 -0.98; p=0.020) per year)

IRR: incidence rate ratio; IRR calculated from Poisson regression

Figure 5.5 Temporal trend in the uptake of HCV treatment adjusted for the factors in Table 5.2

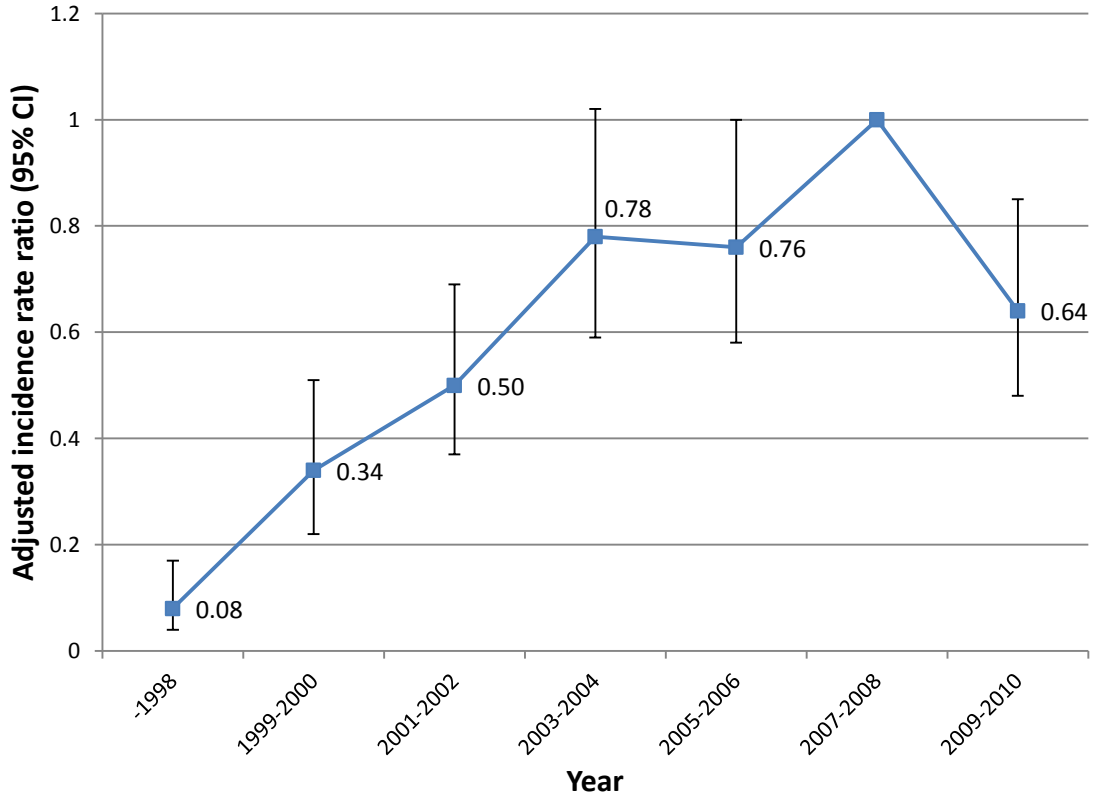


Table 5.2 Poisson regression parameter estimates for factors associated with HCV treatment initiation

		Univariable			*Multivariable*		
Variable		***Estimate***	***95% Confidence Interval***	***P-value***	***Estimate***	***95% Confidence Interval***	***P-value***
Age	Per 10 years	1.02	(0.91 – 1.15)	0.68	0.96	(0.84 - 1.08)	0.49
Male	Vs. Female	1.20	(0.99 – 1.46)	0.070	1.19	(0.97 - 1.47)	0.10
White	Vs. Non-white	0.69	(0.49 – 0.98)	0.037	0.87	(0.60 - 1.28)	0.48
Calendar year: 1998	Vs. 2007-2008	0.04	(0.02 – 0.07)	<0.0001	0.08	(0.04 - 0.17)	<0.0001
1999-2000		0.23	(0.15 – 0.33)	<0.0001	0.34	(0.22 - 0.51)	<0.0001
2001-2002		0.40	(0.29 – 0.54)	<0.0001	0.50	(0.37 - 0.69)	<0.0001
2004-2005		0.67	(0.52 – 0.88)	0.0040	0.78	(0.59 - 1.02)	0.074
2006-2007		0.70	(0.53 – 0.92)	0.0094	0.76	(0.58 – 1.00)	0.049
2009-2010		0.69	(0.52 – 0.91)	0.0093	0.64	(0.48 – 0.85)	0.0021
Started cART *		3.29	(2.54 – 4.26)	<0.0001	1.33	(0.93 - 1.90)	0.12
CD4 <200 cells/mm^3 *	Vs. 200≤ CD4 ≤350cells/mm^3	0.21	(0.14 – 0.32)	<0.0001	0.42	(0.27 - 0.65)	0.0001
CD4 >350 cells/mm^3 *		2.86	(2.35 – 3.48)	<0.0001	1.33	(1.06 - 1.67)	0.013
CD4 unknown *		0.30	(0.18 – 0.51)	<0.0001	1.41	(0.70 - 2.83)	0.34
HIV RNA <500 copies/ml *	Vs. ≥500 copies/ml	3.08	(2.51 – 3.78)	<0.0001	1.39	(1.07 - 1.80)	0.012
HIV RNA unknown *		0.23	(0.16 – 0.33)	<0.0001	1.26	(0.74 - 2.15)	0.40
HCV RNA >800,000 IU/ml *	Vs. 615< HCV RNA ≤800,000IU/ml	1.90	(1.59 – 2.29)	<0.0001	1.21	(1.00 - 1.47)	0.049
South	Vs. West Central Europe	1.17	(0.98 – 0.40)	0.081	1.38	(1.06 - 1.82)	0.019
North		0.74	(0.57 – 0.95)	0.021	1.02	(0.73 - 1.44)	0.89
East Central		1.20	(0.96 – 1.51)	0.12	1.03	(0.74 - 1.43)	0.85

East		1.38	(1.03 – 1.84)	0.030	1.18	(0.78 - 1.78)	0.45
MSM	Vs. IDU	1.48	(1.13 – 1.94)	0.0042	1.36	(1.00 - 1.83)	0.046
Heterosexual		1.17	(0.89 – 1.53)	0.27	1.19	(0.90 - 1.59)	0.22
Other		1.07	(0.76 – 1.51)	0.68	1.25	(0.88 - 1.78)	0.21
HCV genotype 2	Vs. HCV genotype 1	0.75	(0.41 – 1.36)	0.35	1.05	(0.56 - 1.94)	0.89
HCV genotype 3		1.26	(1.04 – 1.53)	0.018	1.20	(0.96 - 1.49)	0.10
HCV genotype 4		1.09	(0.84 – 1.41)	0.51	1.20	(0.90 - 1.59)	0.21
HCV genotype unknown		0.92	(0.72 – 1.19)	0.53	1.02	(0.77 - 1.34)	0.91
HBsAg +	Vs. HBsAg -	0.95	(0.66 – 1.36)	0.77	0.84	(0.58 - 1.21)	0.35
HBsAg unknown		0.28	(0.19 – 0.41)	<0.0001	0.50	(0.33 - 0.76)	0.0012
NR < ALT < 3*NR*	Vs. upper limit NR	2.93	(2.46 – 3.49)	<0.0001	2.33	(1.83 - 2.96)	<0.0001
ALT > 3*NR*		3.39	(2.65 – 4.34)	<0.0001	3.56	(2.61 - 4.86)	<0.0001
ALT unknown*		0.25	(0.20 – 0.31)	<0.0001	1.62	(1.17 - 2.25)	0.0039

ALT: Alanine transaminase; cART: combination antiretroviral therapy; IDU: injecting drug user; MSM: men who have sex with men; NR: normal range for ALT, defined as <50U/L for men and <40U/L for women; 3*NR: 3 times the upper normal range

***Time-updated variable**

In the multivariable model, the calendar year effect mirrored that shown in Figure 5.4 from the univariable analysis.

Figure 5.5 shows that the adjusted incidence of treatment initiation increased until the years 2007-2008 before falling in 2009-2010. Taking 2007-2008 as the reference group, both 2004-2005 and 2006-2007 had slightly lower incidence of HCV treatment uptake (aIRR: 0.78 (95% CI 0.59 – 1.02; *P*=0.074) and 0.76 (0.58 – 1.00; *P*=0.049), respectively) compared with 2007-2008, with borderline statistical significance. The drop in the incidence of HCV treatment uptake in the years after 2007-2008 was also statistically significant (aIRR: 0.64 (0.48 – 0.85; *P*=0.0021)).

5.4.2.1 HCV genotype distribution among treated patients

There was insufficient evidence of a difference in the proportion of individuals treated for HCV according to HCV genotypes. The frequency distribution of treatment initiation among genotypes was G1: 23.6%, G2: 21.2%, G3: 29.5% and G4: 27.8%, with a borderline significant chi-square p-value for differences between the proportions (*P*=0.084). As was shown in Chapter 2 Section 2.2.6 coinfected individuals in the acute phase of HCV infection are treated regardless of HCV genotype because there is evidence that the chance of treatment success is much higher than that observed in chronic infection. Outbreaks of acute HCV among the MSM population have been well documented in Europe[332;339;340;591]. However, removing individuals in the MSM HIV transmission risk group, on the assumption that they were treated during the acute phase of HCV infection, did not change the proportion of individuals treated by genotype (G1:23.2%, G2:20.9%, G3:28.0%, G4:26.2%, *P*=0.29).

The fact that HCV genotype was not associated with HCV treatment uptake is also evidenced by the multivariable Poisson regression model in Table 5.2. In univariable models HCV genotype 3 was associated with treatment initiation (IRR: 1.26 (95% C.I. 1.04-1.53; *P*=0.018) compared to HCV genotype 1), however, when adjusting for other factors there were no significant associations between HCV genotype and treatment uptake.

5.4.2.2 Factors affecting the rate of change in HCV treatment uptake

Interaction terms for categorical covariates were examined to see whether the incidence of HCV treatment uptake varied according to the different levels of the categorical covariates. In particular the interactions between calendar year, HIV transmission risk group and region of EuroSIDA were tested. These interaction terms were individually added to the multivariable model in turn; however, both were non-significant (*P*=0.48 and *P*=0.80, respectively) indicating that there was no evidence to suggest that the incidence of HCV

treatment uptake varied according to HIV transmission risk group or region of EuroSIDA. That the incidence of HCV treatment initiation changed over time in a similar fashion for each of the regions of EuroSIDA is shown in Figure 5.6. The increasing incidence of treatment is noticeable for all regions with a period of tapering off towards the end of the follow-up period. The most obvious difference between regions is seen for the South and West, where the South is constantly above the West, except for 2004, while the tapering off appears to occur two years earlier in the West. This Figure provides further insight into the difference in incidence of treatment uptake between South and West of Europe identified by the Poisson regression model. Further, Northern Europe appears to be the only region yet to experience a drop in the uptake of treatment.

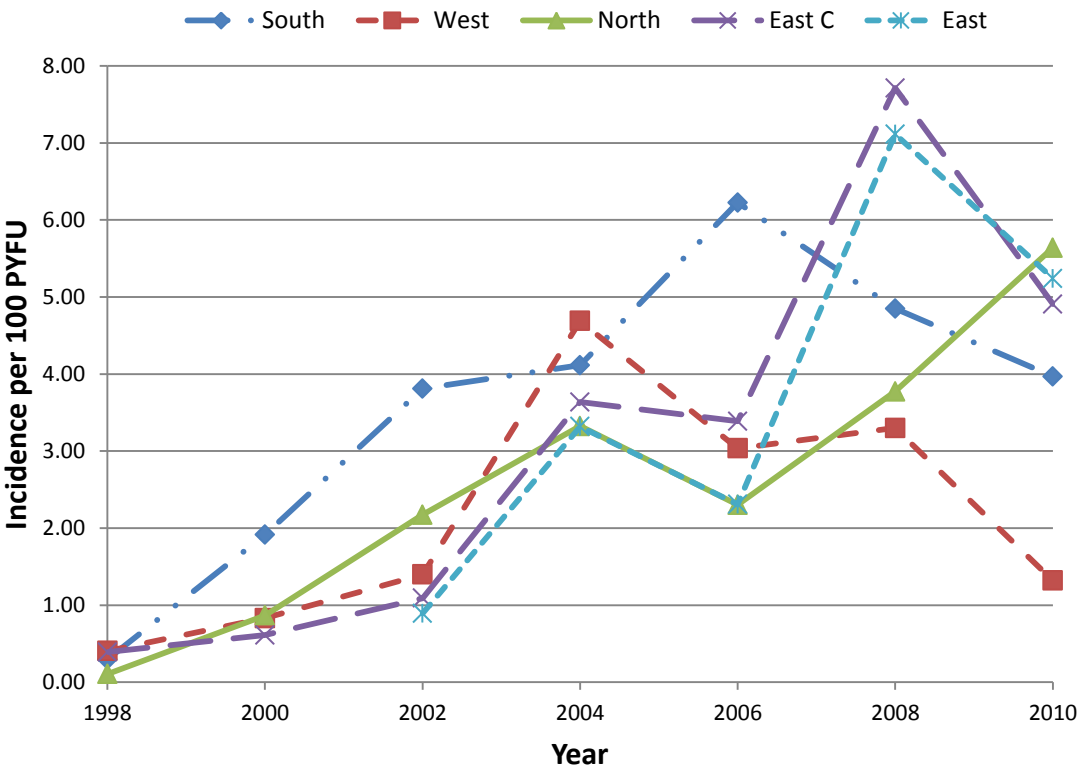
5.4.3 Liver fibrosis levels among treated and untreated patients

Liver biopsy, Fibroscan® and liver fibrosis biomarker data were available for 800 of the 1,984 (40.3%) individuals included in this study. In a multivariable logistic regression model adjusted for the factors listed above, individuals who did not have fibrosis data available were more likely to be MSM (adjusted odds ratio (aOR): 1.53 (95% C.I. 1.07 – 2.18; *P*=0.019) vs. IDUs), reside in Northern Europe (aOR: 1.54 (1.12 – 2.13; *P*=0.0007) vs. Western Europe) and had lower CD4 cell counts at treatment or last follow-up (aOR: 0.72 (0.67 – 0.79; *P*<0.0001) per doubling), than those for whom fibrosis levels could be determined.

Table 5.3 summarises the level of fibrosis among treated patients prior to treatment initiation and at the time of last available follow-up for those yet to be treated. Among individuals with liver biopsy or Fibroscan® data available, a similar proportion of treated patients and those yet to be treated for HCV had ≥F2 fibrosis (43.9% vs. 40.8%, respectively; *P*=0.65). In individuals with HA measured, median HA levels were higher among treated individuals (41.2 ng/mL vs. 28.4ng/mL, respectively; *P*=0.015) and a higher proportion also had HA >100ng/mL (25.5% vs. 12.3%, respectively; *P*=0.011). In individuals with data available to calculate the APRI score, there was no difference between the median APRI score of those treated for HCV and those yet to be treated (0.78 vs. 0.94, respectively; *P*=0.63), nor was there a difference between the proportion of individuals with APRI scores >1.5 (24.1% vs. 29.0%, respectively; *P*=0.55).

When fibrosis was defined using any of the above markers, a higher proportion of individuals treated for HCV were found to have significant fibrosis (≥F2) compared to those yet to be treated (36.0% vs. 22.0%; *P*=0.0003). In multivariable analysis, additionally adjusting the model for predictors of starting HCV treatment in Table 5.2, individuals with

Figure 5.6 Incidence of HCV treatment uptake by region of EuroSIDA



significant current liver fibrosis were 60% more likely to start treatment for HCV than those with <F2 fibrosis (aIRR: 1.60 (95% C.I. 1.14 – 2.25; *P*=0.0065)). However, it is important to note that the majority (64.0%) of individuals with fibrosis data available who started HCV treatment did not have significant fibrosis at the time of their latest fibrosis measurement (median 5.7 months prior to treatment (IQR 2.7 – 11.9)). Furthermore, a large proportion of untreated individuals had significant fibrosis and should, therefore, have been considered for treatment (22.0%). Figure 5.7 shows that many coinfected individuals in Europe appear to remain untreated despite evidence of having significant liver fibrosis. The proportion of people with fibrosis that remain untreated is shown by geographical region in those with fibrosis data available. Southern (23.0%), West Central (15.3%), Northern (31.1%) and East Europe (14.3%) all have a large proportion of coinfected individuals with significant liver fibrosis yet to be treated for HCV. On the other hand, Southern (13.9%), East Central (14.7%) and East Europe (12.7%) all have a large proportion of individuals who have started HCV treatment in the absence of significant liver fibrosis.

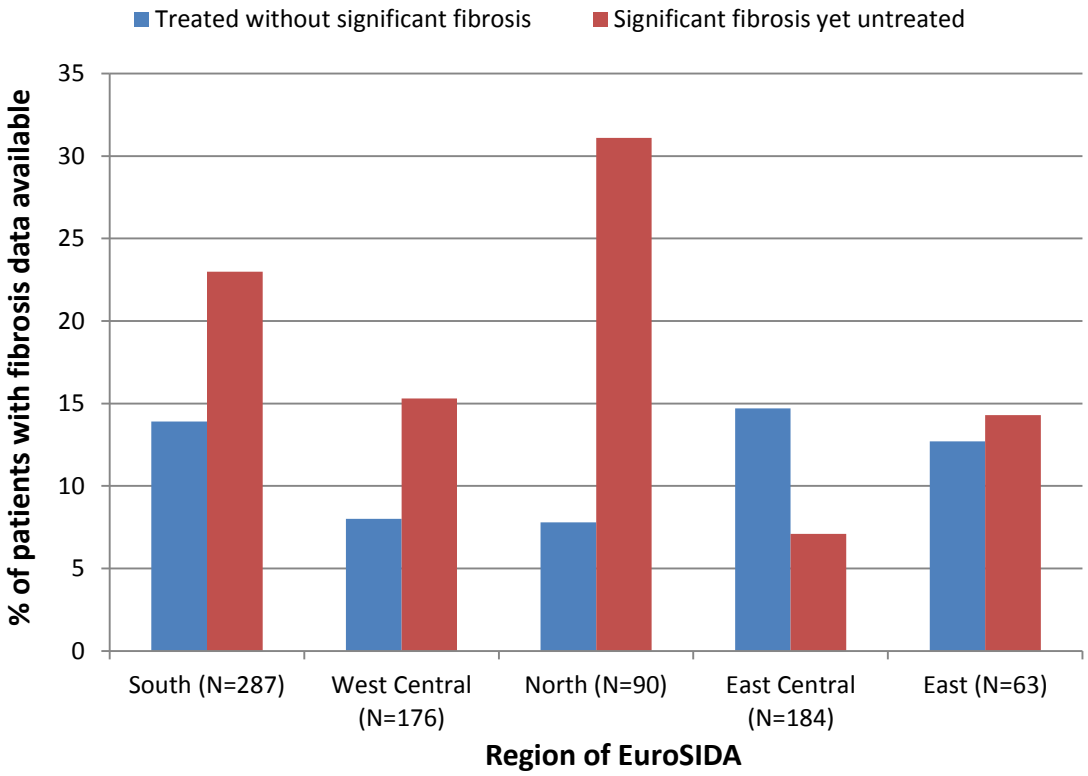
In multivariable analysis on this subset of people with fibrosis data available, extending the analysis shown in Table 5.2 by adding a significant fibrosis covariate and an interaction term between significant fibrosis and region of EuroSIDA, individuals with significant fibrosis were found to be less likely to be treated for HCV in Southern Europe (aIRR: 0.37

Table 5.3 Liver fibrosis markers prior to HCV treatment and last follow-up

Fibrosis Marker		***Treated (N=501)***	***Untreated† (N=1154)***	***P-value***
Any marker	n (%)	150 (29.9)	650 (56.3)	
	Significant fibrosis*	54 (36.0)	143 (22.0)	0.0003
Fibroscan/biopsy	n (%)	66 (13.2)	184 (15.9)	
	<F2	37 (56.1)	109 (59.2)	0.65
	≥F2	29 (43.9)	75 (40.8)	
Hyaluronic Acid	n (%)	47 (9.4)	488 (42.3)	
	Median (IQR)	41.2 (23.4 - 106.9)	28.4 (15.2 - 59.9)	0.015
	>100ng/ml	12 (25.5)	60 (12.3)	0.011
APRI	n (%)	54 (10.8)	62 (5.4)	
	Median (IQR)	0.78 (0.48 - 1.47)	0.94 (0.46 - 1.72)	0.63
	>1.5	13 (24.1)	18 (29.0)	0.55
Time prior to treatment/last follow-up that fibrosis measurement was taken	Median (IQR), months	5.7 (2.7 – 11.9)	57.1 (12.2 – 110.9)	<0.0001

APRI: AST to platelet ratio index; †Untreated patients that are alive at last follow-up; *Significant fibrosis defined using a combined definition of ≥F2 fibrosis from Fibroscan/biopsy, HA >100ng/ml and APRI >1.5

Figure 5.7 Proportion of patients who have been treated for HCV without significant fibrosis or have yet to be treated and have significant fibrosis by region of EuroSIDA



(95% C.I. 0.17 – 0.82; *P*=0.014)) and Northern Europe (aIRR: 0.32 (0.11 – 0.97; *P*=0.044)), compared to those with significant fibrosis in Western Europe.

5.4.4 Completion of full HCV treatment duration

As mentioned in the introduction to this chapter, treatment duration of interferon-based therapy for HCV depends on HCV genotype and is often not well tolerated. The median duration of HCV treatment along with the percentage of individuals who completed at least 80% of the full treatment duration, is shown in Figure 5.8 by HCV genotype. Excluding individuals with unknown HCV genotype, 271 individuals out of 416 (65.1%) completed at least 80% of the full treatment duration. The longest median HCV treatment durations were seen for HCV genotypes 1 and 4 (46.8 and 45.8 weeks, respectively), in which treatment guidelines recommend the longest treatment duration. However, the highest proportions of individuals completing at least 80% of the full treatment duration were seen for HCV genotypes 2 and 3 (72.7% and 80.9%, respectively). Treatment completion rates among the more difficult to treat HCV genotypes 1 and 4 were just over a half (57.7% and 52.9%, respectively).

Figure 5.8 Percentage of patients completing the full duration of HCV treatment and the median duration of treatment by HCV genotype

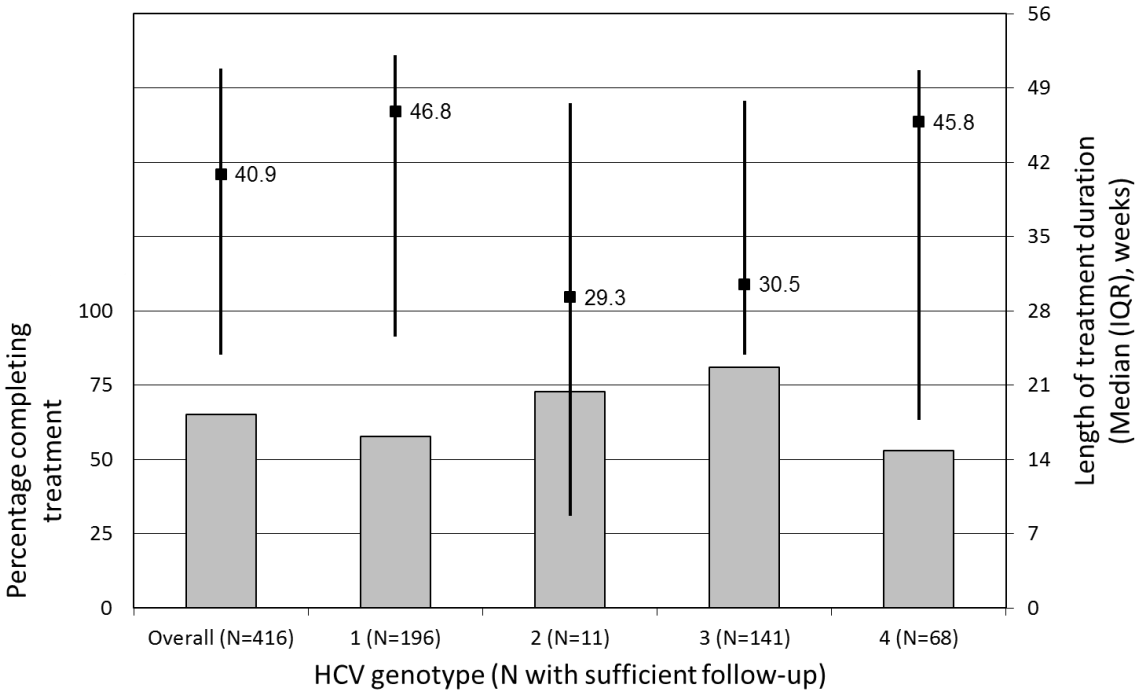


Table 5.4 shows multivariable logistic regression odds ratios for factors associated with completing at least 80% of the full duration of treatment. Individuals who are younger (aOR: 0.70 (95% C.I. 0.50 – 0.98; *P*=0.038) per 10 years), with HCV genotypes 2 or 3 (aOR: 3.31 (1.92 – 5.70; *P*<0.0001) vs. HCV genotype 1) and HIV-RNA <500copies/ml at treatment initiation (aOR: 2.25 (1.22 – 4.17; *P*=0.0095) vs. HIV-RNA ≥500 copies/ml), were more likely to complete the full duration of HCV treatment. There was also borderline statistical significance to suggest that individuals with CD4 cell counts <200 cells/mm^3 at the time of HCV treatment initiation were more likely to complete therapy (aOR: 3.24 (0.99 – 10.59; *P*=0.051) vs. CD4 cell count between 200 and 350 cells/mm^3).

Of the 416 individuals with known HCV genotype, 44 (10.6%) discontinued their treatment before completing 12 weeks of therapy (G1: 8.7%, G2: 27.3%, G3: 10.6%, G4:13.2%). In a multivariable logistic regression model similar to that shown in Table 5.4, but with the endpoint of discontinuing HCV treatment before 12 weeks of therapy, baseline use of cART was the only factor significantly associated with early treatment discontinuation. In particular, those not taking cART at treatment initiation were at 4-fold increased risk of discontinuing HCV treatment before reaching 12 weeks of therapy (aOR: 3.85 (95% C.I. 1.35 – 11.11; *P*=0.12)) compared to those taking cART at HCV treatment initiation.

Table 5.4 Multivariable logistic regression factors associated with completion of a full duration of HCV treatment

Parameter		***Odds Ratio***	***95% Confidence Interval***	***P-value***
Age	Per 10 years	0.70	(0.50 - 0.98)	0.038
Male	Vs. Female	1.25	(0.73 - 2.14)	0.42
White	Vs. Non-white	0.93	(0.36 - 2.41)	0.88
MSM	Vs. IDU	1.09	(0.49 - 2.43)	0.84
Heterosexual		1.70	(0.80 - 3.61)	0.17
Other		1.37	(0.57 - 3.26)	0.48
South	Vs. West Central Europe	0.55	(0.27 - 1.12)	0.10
North		0.47	(0.20 - 1.14)	0.094
East Central		1.32	(0.54 - 3.24)	0.54
East		0.85	(0.27 - 2.65)	0.78
Baseline cART		1.37	(0.78 - 2.43)	0.27
Prior AIDS diagnoses		0.65	(0.31 - 1.37)	0.26
CD4 <200 cells/mm^3 *	Vs. 200 ≤ CD4 cells/mm^3 ≤ 350	3.24	(0.99 - 10.59)	0.051
CD4 >350 cells/mm^3 *		1.30	(0.73 - 2.31)	0.36
HCV genotype 2/3	Vs. HCV genotype 1	3.31	(1.92 - 5.70)	<0.0001
HCV genotype 4		0.68	(0.36 - 1.27)	0.23
HCV RNA > 800,000 IU/ml*	Vs. 615 < HCV RNA IU/ml ≤ 800,000	1.15	(0.69 - 1.94)	0.59
HIV RNA < 500 copies/ml*	Vs. HIV-RNA ≥ 500 copies/ml	2.25	(1.22 - 4.17)	0.0095
Calendar year*	Per year	0.99	(0.91 - 1.07)	0.82

***At the time of HCV treatment initiation**

5.5 Discussion

Uptake of HCV treatment

This chapter describes the rate of uptake of HCV treatment among HIV-positive individuals chronically infected with HCV. One of the main findings of this analysis is that 25% of coinfected individuals with viremic HCV infection were treated with interferon-based therapy for HCV infection in EuroSIDA between 1998 and 2010. This is a higher prevalence of treatment for HCV than has been reported in previous work on the topic where treatment uptake has typically been very low. Studies from around the beginning of 2000 from *Fleming et al*[444] and *Fultz et al*[584] found that eligibility for HCV treatment among HIV/HCV coinfected individuals was low, citing missed clinic visits, psychiatric illness and a high rate of alcohol abuse as barriers to treatment.

In particular, *Fultz et al* reported that due to these contraindications just 3% of the study population of coinfected individuals went on to receive interferon-based treatment[584]. A more recent study from 2005 by *Rauch et al* also found very low eligibility for interferon-based treatment among coinfected individuals[443]. The authors reported that 77% of coinfected individuals in the Swiss HIV Cohort were ineligible due to low CD4 cell counts or on-going illicit drug use. However, even among those eligible for treatment, uptake of therapy remained low with 62% rejecting treatment as they were fearful of side-effects, leading to an overall treatment uptake of just 8%[443].

One of the strengths of this study is that each individual's HCV status was characterised using HCVAb and HCV RNA, including a large number of individuals over a long period of follow-up. In the absence of HCV RNA data, the proportion of individuals selected for treatment is likely to be underestimated as clinicians would only consider treating individuals with active on-going HCV viral replication. If it is unknown whether individuals are positive for HCV RNA or they have cleared the virus spontaneously, the denominator for the proportion of individuals treated is typically inflated by the inclusion of individuals who would not be considered for treatment.

This may potentially explain the discrepancy in frequency of treatment initiation observed in the present analysis and that estimated in previous analysis of EuroSIDA data. In 2006, *Mocroft et al* report on limited but increasing incidence of treatment for HCV in EuroSIDA, classifying individuals as HCV positive on the basis of an HCVAb test but not an HCV RNA measurement. The authors reported the proportion of treated individuals to be just 7.6%, but also documented a 38% increase in the incidence of treatment over time and up to 2004[582]. It would seem from the current analysis that this increase in the incidence of

treatment year-on-year has continued until 2007/08 and accounts partially for the difference between the results of the two studies.

Another recent European study by the *Cohere collaboration,* with data to December 2009, reported an uptake of treatment of 12% among 6,433 coinfected individuals[583]. However, this study only had HCV RNA data available for 12% of the study population and was geared towards looking at the effect of HCV treatment on short-term CD4 cell count changes and the long-term effect on overall mortality, rather than specifically documenting the rate of HCV treatment uptake. The study included a median follow-up of 72 months (IQR: 39 – 108) per individual, which is far fewer than the 168 months (IQR: 121 – 204) per individual that were included in the current study, and goes some way to explaining the differences in treatment uptake between the two studies.

By fitting a univariable Poisson regression model, I found that the uptake of HCV treatment increased by 27% per year between 1998 and 2007, before falling by 12% per year between 2007 and 2010. In multivariable analysis, accounting for the demographic differences in the study population, a similar increase in treatment uptake was described from 1998 to 2007/2008 with a significant drop in treatment uptake in 2009/2010.

The increasing uptake of HCV treatment most likely reflects improvements made in controlling HIV infection and the introduction of peg-IFN. Peg-IFN introduced a longer lasting interferon treatment with a longer circulatory time, leading to improved SVR rates for treatment of HCV. In 2004, *Chung et al* reported the results of a clinical trial showing that although SVR rates remained modest for HCV genotype 1, they were significantly improved for other HCV genotypes when using peg-IFN with ribavirin compared with standard interferon plus ribavirin, indicating that 41% and 12% of treated individuals achieved SVR, respectively[435].

In another clinical trial of HIV/HCV coinfected individuals from the same year, *Carrat et al* also reported a significant increase in SVR with the use of peg-IFN plus ribavirin compared to standard interferon plus ribavirin (27% and 20%, respectively), noting that the difference was more marked for HCV genotypes 1 and 4[434]. Further, studies have since gone on to document the effect of baseline CD4 cell count on the effectiveness of treatment based on peg-IFN[567], and the comparative efficacy of different types of peg-IFN[448].

While the introduction of peg-IFN is the most likely explanation for the increase in HCV treatment uptake up to 2007, the decreases in treatment uptake seen after 2007 have multiple possible explanations. It is possible that there has been treatment saturation of the

easy-to-treat individuals eligible for therapy, meaning the pool of individuals with the potential to be treated has diminished over time. However, EuroSIDA is not a closed cohort and new individuals added after 2007 could add to the pool of individuals eligible for treatment.

Another explanation is that clinicians are waiting for new potent treatments to come to market. As described in the introduction of this chapter, the first generation of DAAs for HCV became available in 2011[439] with expected SVR rates of approximately 75% in patients with HCV genotype 1[437;440]. Because a greater chance of HCV treatment success was associated with younger age, low HCV RNA (<800,000IU/ml) and non-HCV genotype 1[554], clinicians and their patients in EuroSIDA in the years 2009/2010 with low fibrosis stages, high HCV RNA or HCV genotype 1 may have felt that they should wait for the more efficacious HCV therapy to be licenced for use in the clinic [592], therapies which also might avoid the unpleasant side effects associated with treatment with interferon[593].

Factors associated with starting HCV therapy

Coinfected individuals initiating HCV therapy in this analysis were more likely to have CD4 cell counts greater than 350 cells/mm^3, ALT values above the normal range and HIV RNA below 500 copies/ml, which is in line with current treatment guidelines to treat HIV infection first to attain immune-sufficiency before initiating HCV treatment[1]. High HCV RNA levels (greater than 800,000 IU/ml) were also associated with treatment uptake, even though high HCV RNA has been identified as a predictor of poor treatment response[554]. Interestingly, individuals residing in Southern Europe compared with Western Europe and those belonging to the MSM HIV transmission risk group compared with the IDU risk group, were also more likely to be treated.

One potential explanation for the differences between European region and mode of HIV transmission with respect to HCV treatment uptake, could be the HCV genotype distribution, however, HCV genotype does not appear to explain the differences reported here. A similar proportion of patients were treated among each of the HCV genotypes and HCV genotype was included in the multivariable model to identify factors associated with HCV treatment uptake.

The higher rate of treatment in Southern Europe could have a variety of explanations. A higher prevalence of HCV infection in that region may have led to greater clinician experience in dealing with HIV/HCV coinfection. Further, differences between national treatment guidelines and local traditions will play a role in determining the rate of treatment from region to region. Treatment uptake may be expected to be lower in Eastern Europe

compared to other regions but was not the case in this analysis. This may be due to the inclusion criteria employed in this study. Many individuals from the Eastern region were excluded from the study as both HCVAb and HCV RNA positive test results were required and many individuals residing in Eastern Europe did not have available HCV RNA.

The most likely explanation of the higher rate of HCV treatment uptake seen among MSM is the recently reported outbreak of acute HCV in this population in Europe. *Rauch et al* from the Swiss HIV cohort recently reported that among HIV-positive individuals unsafe sex was associated with an increased risk of HCV seroconversion, and that an increase in the prevalence of HCV in this population was most likely caused by sexual transmission[591]. V*an Der Laar et al* also reported on the emergence of an MSM-specific HCV transmission network in Amsterdam in 2006, suggesting that HIV-positive MSM with high-risk sexual behaviours were at increased risk of HCV infection[339].

Treatment for acute HCV infection is associated with far better cure rates than chronic HCV infection, regardless of HCV genotype[594;595], which could lead to preferential treatment in this group. Further, MSM are also often considered to be easier to treat and more adherent to therapy than IDU, who are often not considered for treatment due to on-going drug abuse[443]. Unfortunately, data on active drug abuse was added to EuroSIDA in 2012 after the follow-up period of this study. Therefore, controlling for differences between the active drug users across regions of EuroSIDA was not possible.

Fibrosis levels in treated and untreated individuals

Among those who had available fibrosis marker data, a higher proportion of individuals treated for HCV infection had significant fibrosis prior to treatment, as evaluated using liver biopsy, Fibroscan, HA and the APRI score, compared with those yet to receive treatment for HCV at last follow-up. In a sensitivity analysis, significant fibrosis (≥F2 fibrosis as defined by the METAVIR scoring system[420]) was associated with a 60% increased incidence of treatment uptake. However, looking closely at the number of individuals with fibrosis I noticed that although those with fibrosis are more likely to be treated, just one-third of individuals treated for HCV infection were known to have significant fibrosis. Further, 22% of individuals yet to receive treatment for HCV did have significant liver fibrosis and should have been considered for treatment for HCV.

Fibrosis data was included up to 2-years prior to treatment in this analysis; therefore it is possible that some individuals may have progressed to significant fibrosis in this time. However, the median time prior to initiating HCV treatment that fibrosis data was available was 5.7 months and liver fibrosis progression rates have been shown to be slow in this

patient population[389]. The median time prior to last follow-up that fibrosis data was measured in those yet to be treated for HCV was 57 months, meaning that it is far more likely that fibrosis progression would have occurred in untreated individuals prior to last follow-up and that if anything the analysis is likely to be underestimating the level of fibrosis in individuals yet to be treated for HCV.

In a multivariable model, individuals with significant fibrosis were also found to be less likely to be treated in Southern and Northern Europe compared to those with significant fibrosis in Western Europe. Although the fibrosis data were not complete, this finding is likely to some degree to be due to unmeasured confounding where there is contraindication to treatment. Of the individuals with significant fibrosis that have yet to be treated for HCV, 77% were IDU, which is often associated with contraindications to therapy[443]. However, it is clear that there needs to be renewed focus on correctly identifying eligible patients for treatment for HCV.

Duration of HCV treatment

The median duration of HCV treatment was close to the guideline minimum duration of therapy for HCV genotypes 1 and 4 and surpassed it for genotypes 2 and 3. However, the rate of discontinuation before completing the full duration of therapy was high, with one-third of all patients who started HCV therapy discontinuing before the end of treatment.

The lowest rates of treatment completion were seen for HCV genotypes 1 and 4 which reflects the heightened difficulty in treating these individuals, with anticipated lower response rates and treatment discontinuation more likely when following response-guided therapy[1;450]. Further, in adjusted logistic regression, younger age, HCV genotype 2 or 3 and HIV RNA <500 copies/ml at HCV treatment initiation, were found to be independently associated with increased likelihood of treatment completion. In addition, initiating cART early during clinical care was associated with a lower rate of early HCV treatment discontinuation (before 12 weeks of therapy). Younger age and HCV genotype 2 or 3 are well-known predictors of HCV treatment success[439], while controlling HIV infection with cART, lowering HIV RNA and raising CD4 cell counts prior to initiating HCV therapy is best practice to ensure the highest chance of completing the full treatment duration, in line with current HCV treatment guidelines[1;450].

5.5.1 Limitations

The main limitation of this study was that HCV RNA data was not available for a large proportion of HCVAb-positive individuals. Individuals that have cleared HCV spontaneously without treatment would not be considered for HCV therapy, as they are already cured, so

this information is vital to the study. Consequently, a large number of HCVAb-positive individuals were excluded from Eastern Europe where HCV RNA data is not as frequently documented, despite the fact that in the absence of treatment >75% are likely to be chronically infected. Therefore, the results presented here are likely to be less applicable to Eastern Europe.

Another limitation of this study is that in clinical practice many individuals who initiate HCV therapy will stop treatment early due to severe adverse events; unfortunately these are not recorded in EuroSIDA and cannot be analysed. This might have resulted in an underestimation of the rate of treatment discontinuation prior to completing a full course of therapy. Further, coinfected individuals may have other contraindications to therapy which are not recorded (unmeasured confounding factors) and clinicians will most likely use their own judgment when selecting who to treat.

Liver fibrosis levels were estimated using a combined definition including liver biopsy, Fibroscan® measurements as well as the non-invasive biomarkers HA and the APRI score. Although the use of these biomarkers has been well described and widely reported in other studies, they will be less precise than biopsy at determining liver fibrosis.

5.5.2 Conclusions

The analysis in this chapter documented an increase in the incidence of treatment for HCV infection in EuroSIDA from 1998 to 2010. In multivariable analysis adjusting for a number of measured potential confounding factors, increasing incidence of treatment was found to have peaked in 2007/2008. This result probably reflects the introduction of peg-IFN, which offers a longer lasting circulatory time and increased chance of SVR. This increase was followed by a plateau in the incidence of treatment uptake which may have a larger number of explanations, including the fact that most clinicians are reluctant to start current regimens knowing that more efficacious and better tolerated drugs will be soon available.

Individuals who were selected for HCV treatment were mostly aligned with current treatment guidelines, with well controlled HIV infection and high CD4 cell counts. However, there were important deviations from the guidelines referring to the level of liver fibrosis among treated individuals, with as many as two-thirds of patients treated for HCV lacking significant liver fibrosis which indicates treatment.

Further, the results have also shown that younger age, HCV genotypes 2 or 3 and undetectable HIV RNA at the time of HCV treatment initiation were all associated with an increased likelihood of completing a full duration of therapy. In the opening of the results in

this chapter the HCV treatment prevalence is documented as 25% among HIV/HCV coinfected individuals in EuroSIDA. Although this figure is higher than in previous studies there remain individuals, including some with significant liver fibrosis, who have yet to be exposed to HCV treatment and emphasis should be placed on continuing to promptly diagnose people with HCV infection and identify those in need of HCV treatment.

Chapter 6

The incidence of antiretroviral drug discontinuation among HIV/HCV coinfected patients and those with significant liver fibrosis

6.1 Introduction

Antiretroviral treatment (ART) for HIV infection has led to a dramatic decrease in AIDS-related mortality and morbidity[509;596-599]. In some countries liver end-stage related disease is now the leading cause of death among HIV-positive individuals due to on-going hepatitis C virus (HCV) coinfection[600]. Coinfection with HIV and HCV is common due to shared transmission routes and in Europe coinfection rates among HIV-positive individuals are estimated to range from 20% in Central and Northern regions to 50% in Southern and Eastern regions[404]. Although great strides have been made in the management of HIV/HCV coinfected individuals, longer life expectancy has led to more individuals presenting with hepatic and renal impairment requiring treatment, consequently pharmacokinetic considerations are now central to appropriate antiretroviral (ARV) dosing strategies[601]. Therefore, analysis of ARV drug discontinuation among HIV/HCV coinfected individuals is important to identify those with inappropriate HIV treatment regimens.

6.1.1 The cytochrome P450 enzyme system

Cytochrome P450 (CYP450) enzymes are essential for the production of cholesterol, steroids, the detoxification of foreign chemical and the metabolism of drugs[602;603]. The CYP450 enzyme group is named as such because they are bound to membranes in a cell (*cyto*) and contain a heme pigment (*chrome and P*) that absorbs light at a wavelength of 450nm when exposed to carbon monoxide[603]. The term CYP450 covers a wide range of more than 50 individual enzymes, however, the CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A5 enzymes are responsible for metabolising 90 percent of drugs, the two most significant enzymes being CYP3A4 and CYP2D6[603-605]. While these enzymes are predominantly found in the liver, they can also occur in the small intestine, lungs and kidneys[605].

Many drug interactions are a direct result of a change in CYP450 metabolism, while drugs interact with CYP450 in many ways[603;606]. Some drugs are metabolised by just one enzyme

and others by multiple enzymes[607]. Drugs that interfere with CYP450 metabolism are called either inhibitors or inducers. For example, ordinary grapefruit juice is known to be an inhibitor of CYP3A4, consequently drugs such as diazepam (Valium), which has a metabolic contribution from CYP3A4, will be available in the blood stream for longer if grapefruit juice is taken at the same time[603;608]. These interactions, via inhibitors and inducers, can often cause standard drug doses to lead to adverse effects as they can lead to longer than intended expose to drugs in the body[603;609].

6.1.2 Antiretroviral drug metabolism

Most ARV drugs are metabolised in the liver by the hepatic CYP450 enzyme system or removed from the body via renal excretion, often with metabolising contributions from both the liver and the kidneys[601]. Most nucleoside reverse transcriptase inhibitors (NRTI) are water soluble and relatively well absorbed by the body so are broken down and excreted via the kidneys[601;610]. However, abacavir, zidovudine and didanosine all undergo extensive hepatic metabolism by alcohol dehydrogenase, glucuronidation, and purine nucleoside phosporylase (PNP), respectively[610]. Although these mechanisms are not via CYP450, drug interactions can still occur that interfere with these processes[610].

The non-nucleoside reverse transcriptase inhibitor (NNRTI) drug class on the other hand is predominantly metabolised by the CYP450 enzyme system, while individual drugs play the role of inducer or inhibitor to various CYP enzymes[601;611]. Therefore, hepatic impairment is likely to affect NNRTI pharmacokinetics and metabolism. Similarly, protease inhibitors (PI) have complex effects on CYP activity, being extensively metabolised by the liver. In addition, in most cases the current standard of care recommends using ritonavir as a booster to a partner PI[1;601]. Ritonavir is known to be a powerful inhibitor of CYP3A4 and slows down the metabolism of PIs considerably, leading to higher blood plasma concentrations of the boosted PIs[601;612].

As many ARV drugs are metabolised in the liver advanced hepatic disease as a result of HCV infection could be considered a risk factor for ARV drug discontinuation and drug-related toxicity. Liver damage and metabolic impairment have the potential to interfere with the pharmacokinetic processes of the NNRTI and PI drug classes in particular.

6.1.3 HCV coinfection and ARV-related hepatotoxicity

Hepatotoxicity is defined by elevation of the liver transaminases alanine transaminase (ALT) and aspartate transaminase (AST)[613]. The AIDS Clinical Trials Group have proposed a standardised grading system for changes in ALT and AST relative to the upper limit of the

normal range (ULN), ranging from grade 0 (<1.25 x ULN) to grade 4 (>10 x ULN)[613]. Grades 1 and 2 represent mild hepatotoxicity with grades 3 and 4 considered to be severe hepatotoxicity[613].

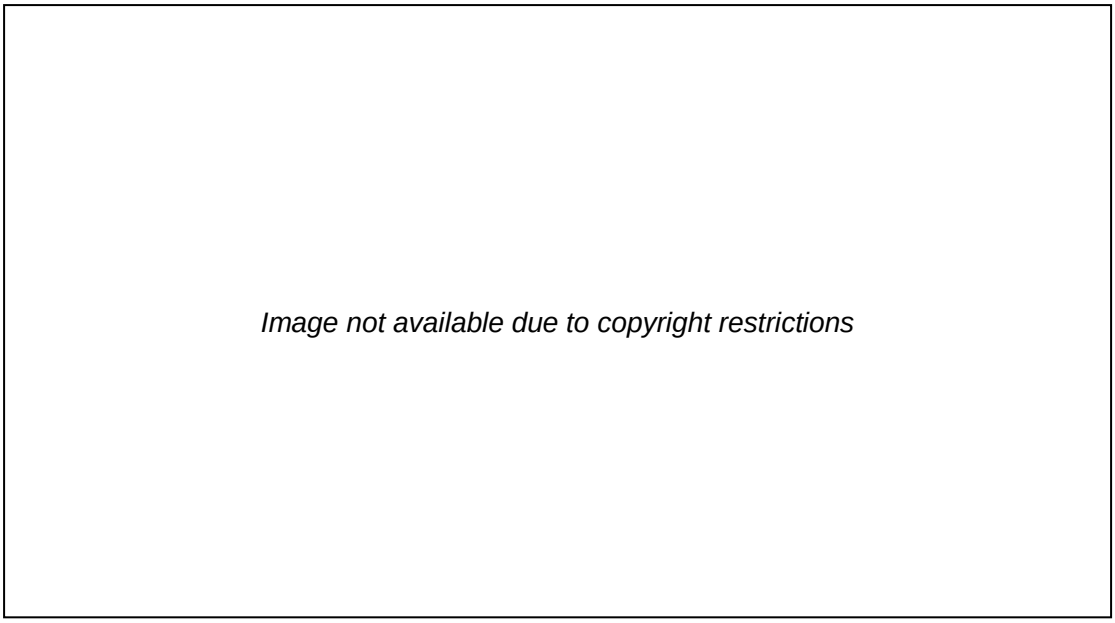
In 2000, *Sulkowski et al* described the incidence of severe hepatotoxicity events among 298 patients prescribed ART, 154 positive for HCVAb. The authors found that hepatotoxicity of any grade was seen in 54% of individuals coinfected with HIV/HCV compared to 39% of those with HIV monoinfection (*P*=0.009)[536]. However, the authors also state that 88% of individuals with HCV or HBV infection did not experience toxic effects of ART and that coinfected individuals did not need to avoid the use of any particular drug class[536].

Many studies have since confirmed these findings[614;615], with some studies specifically focusing on the role of HCV and HBV coinfection. *Den Brinker et al* studied liver enzyme elevations (LEE), defined as five times the ULN and absolute increases of over 100U/l, in 394 patients of whom 7% were HBsAg positive and 14% were HCVAb positive. The authors reported that LEE were more frequent among those coinfected with hepatitis and that after adjustment for baseline transaminases the presence of HCVAb was associated with 146% increased risk of LEE compared to HIV monoinfected individuals[534]. However, the authors also stated that although LEE was more common in coinfected individuals it was not necessary to modify antiretroviral therapy in these patients[534].

In 2001, *Aceti et al* performed a retrospective study of 1,325 HIV-positive individuals treated with ART for at least 6 months, with the intention of studying the occurrence of hepatotoxicity in those receiving ART specifically containing PIs[616]. The authors reported that there were significantly higher levels of hepatotoxicity among coinfected individuals and in logistic regression viral hepatitis coinfections were independent risk factors for hepatotoxicity[616]. After six months of treatment the authors found that ritonavir was the ARV most strongly associated with hepatotoxicity, with higher rates of severe hepatotoxicity seen in the coinfected group[616]. The authors noted that among HIV monoinfected individuals there were no differences in the occurrence of hepatotoxicity with different PIs[616].

In 2005, *Nunez* reviewed the subject of hepatotoxicity and ART, summing up that liver toxicity is important since it can often lead to ARV discontinuation, particularly in HIV-positive individuals with HCV or HBV coinfection[617]. The review also emphasises that the incidence of drug-induced liver toxicity is not well known for most antiretrovirals and that it is difficult to determine the role of specific drugs in a combination of treatments[617]. Despite

Figure 6.1 Mechanisms of hepatotoxicity linked with antiretroviral therapy[617]



the published studies, questions remain about the mechanisms involved in the development of hepatotoxicity and the process is thought to be multifactorial. *Nunez* suggested that the development of raised liver transaminases was potentially dependent upon a number of factors, including HCV or HBV coinfection, ART, methadone therapy, alcohol use and the development of steatosis, Figure 6.1[617].

These studies have provided important information but they are subject to a number of important limitations. First, most were based on a relatively low number of individuals, with a small proportion coinfected with HCV. Second, all relied on HCVAb in order to define HCV infection. As discussed in Chapter 5 of this thesis, it is not possible to accurately categorise HCV-infected individuals without the use of HCV RNA data to document on-going viral replication. Third, the clinical consequences of the observed raised transaminases in terms of drug discontinuation are not discussed. Finally, information on the level of liver fibrosis among coinfected individuals was not available or was limited in these studies; consequently, the role of HCV viral replication and liver fibrosis in causing ART-related toxicity and drug discontinuation remains unclear.

6.1.4 Impaired liver function as a cause of hepatotoxicity

The *Nunez* study identified steatosis combined with mitochondrial toxicity as one of the potential causes of raised liver transaminases[617]. Steatosis is the first stage in the spectrum of non-alcoholic fatty liver disease (NAFLD), which can progress to severe inflammation with extensive fibrosis or cirrhosis[618]. While hepatitis steatosis without inflammation is thought to have a good prognosis, non-alcoholic steatohepatitis is known to progress to cirrhosis in a number of cases[618]. Given that steatosis has been identified as a potential

cause for raised liver transaminases, the hypothesis follows that further advanced stages of NAFLD will also contribute to hepatotoxicity.

A recent study from 2010 by *Dominguez et al* and the HEPDOSE study compared plasma ARV drug concentrations between matched HCV coinfected individuals and HIV monoinfected individuals[619]. Although only including 73 HIV/HCV coinfected individuals and 66 HIV monoinfected individuals, the authors found that minimum ARV plasma concentrations were higher among coinfected individuals taking NNRTIs compared with HIV monoinfected individuals, especially in those with advanced liver fibrosis[619]. This finding indicates that high levels of liver fibrosis among coinfected individuals may lead to overdosing of ARVs via inhibition of the CYP450 activity[620], which in turn could lead to hepatotoxicity manifested clinically as ARV drug discontinuation[621].

6.1.5 Hyaluronic acid as a marker of liver fibrosis

Historically, liver biopsy has been considered the gold standard for detecting and monitoring the progress of liver fibrosis among HIV/HCV coinfected individuals[622]. However, liver biopsy is an invasive procedure with a small risk of complications and death[623], and repeated biopsy would not be acceptable for most individuals[624;625]. In addition, there is potential for considerable observer bias in the histological staging of a liver biopsy[626]. Therefore, data on liver fibrosis levels has been limited in previous studies.

Non-invasive biomarkers have previously been identified as useful tools in identifying individuals at risk of liver-related complications, such as the AST to platelet ratio index (APRI) (see Chapter 2 Section 2.2.5.3). Among these, hyaluronic acid (HA) has recently been shown to be an accurate marker for liver fibrosis[627]. Importantly, in 2005 *Nunes et al* showed that the diagnostic performance of HA as a marker for liver fibrosis was not affected by HIV/HCV coinfection[628]. In fact the authors found that the diagnostic performance of HA tended to be better among HIV/HCV coinfected individuals compared to HIV monoinfected individuals, however, this was likely due to a small sample size of coinfected individuals[628].

Other studies have focused on determining what is to be considered the normal range for HA and the optimal cut-off points for the histological stages of liver fibrosis. Consensus seems to suggest that the normal range for HA in healthy controls lies between 0-75ng/ml, with any HA measurement above 100ng/ml being indicative of significant hepatic fibrosis (METAVIR score ≥F2)[587;622;628].

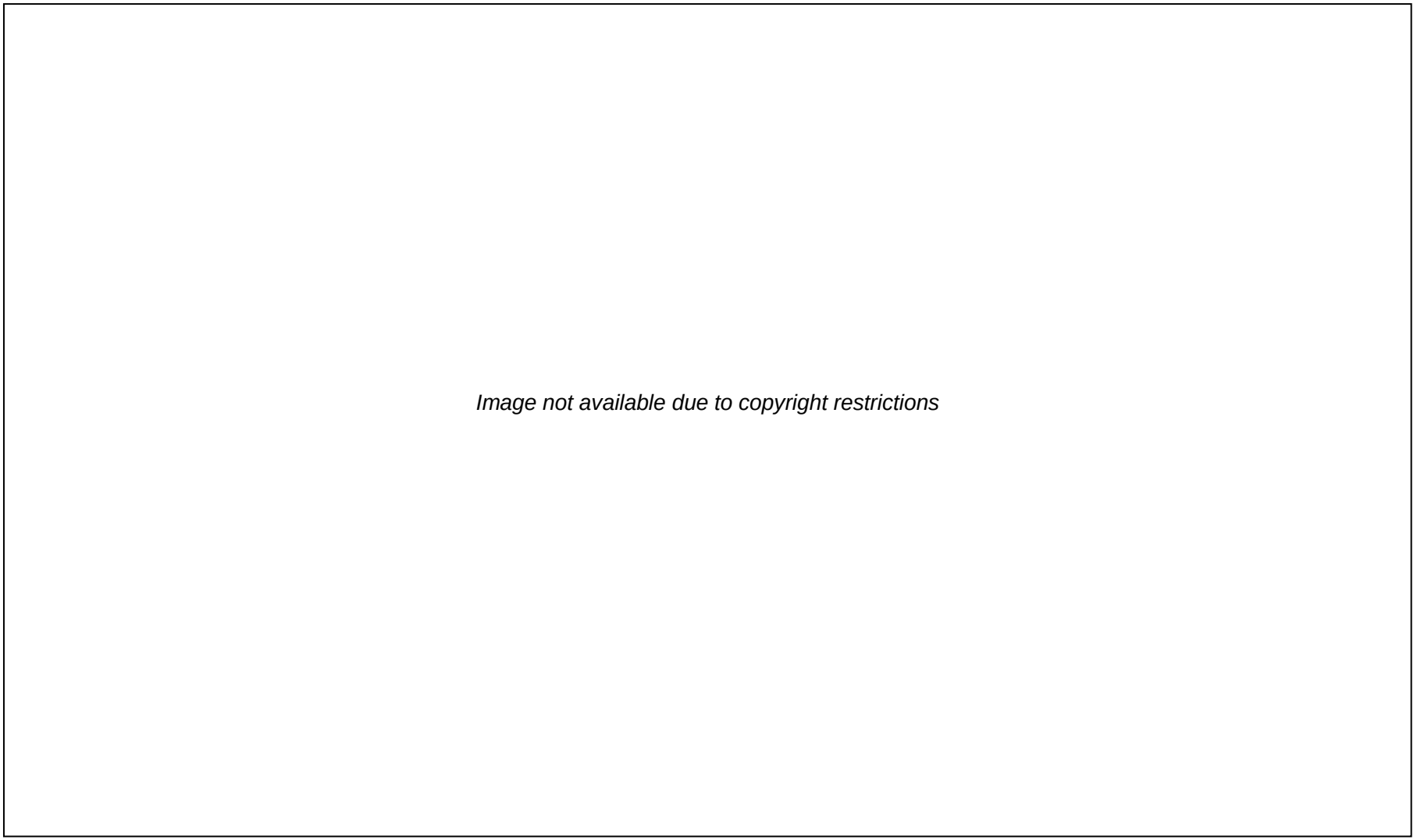
6.1.6 Previous EuroSIDA studies of antiretroviral drug discontinuation

EuroSIDA have previously published analyses investigating ARV drug discontinuation in relation to HIV/HCV coinfection. In 2005, *Mocroft et al* published two papers on the subject[629;630]. The first dealt with reasons for stopping ARV treatment in HIV monoinfected and HIV/HCV coinfected individuals[629]. Including 1,198 individuals (30% HCVAb positive) starting cART after 1999 the authors reported that after one year of treatment 70% of patients remained on their original regimen, 24% switched treatments and 6% stopped treatment all together[629]. The most frequent reason for discontinuation reported was toxicity in 30% of cases, followed by patient or physician choice in 30% of cases[629]. Further, those positive for HCVAb were more likely to discontinue all or part of their cART regimen due to toxicity or patient/physician choice. HCVAb positive individuals also had 46% increased risk of ARV drug discontinuation due to toxicity compared to those without HCV[629].

The second of the two papers focused more specifically on which components of cART were stopped and whether there were specific drug classes that were at higher risk of drug discontinuation[630]. Including 4,929 individuals (28% HCVAb positive) the authors reported over 4,500 ARV drug discontinuations for nucleoside pairs and third drugs (the cART component other than the nucleoside pair)[630]. Table 6.1 shows the estimated incidence of ARV drug discontinuation by exposure group. For nucleoside pairs and third drugs those HCVAb positive had higher rates of discontinuation compared to those HCVAb negative (19.1 per 100 person years follow-up (PYFU) vs. 15.8 and 22.4 vs. 18.4, respectively)[630]. Overall those HCVAb positive were at 21% and 22% higher risk of discontinuing nucleoside pairs and third drugs, respectively[630]. Further, although there were a relatively low number of ARV drug discontinuations due to liver-related toxicity 56 cases in total, they were significantly more frequent among those HCVAb positive compared to those HIV monoinfected (2.3% vs. 0.7%, respectively)[630].

These two previous EuroSIDA studies are important in that they bridge the gap between the scientific understanding of hepatotoxicity and the clinical implications regarding ARV drug discontinuation. They report a substantial rate of drug discontinuation with some 20% of patients stopping their nucleoside pair and third drug each year, while clearly showing that the problem is more pronounced among those HCVAb positive. However, they suffer from some of the same limitations as the previous studies, mainly that HCV status is classified solely on HCVAb body testing and not HCV RNA as an indication of on-going viral replication, and there was no available information on the role of liver fibrosis as a risk factor for ARV drug discontinuation. In addition, the investigators note that it was virtually impossible to disentangle HCVAb status from IDU due to strong co-linearity.

Table 6.1 Incidence of antiretroviral drug discontinuation stratified by hepatitis C status[630]



6.2 Aims

The aims of this chapter were to describe the incidence of ARV drug discontinuation among HIV monoinfected and HIV/HCV coinfected individuals in EuroSIDA, in order to give insight into the relationship between hepatotoxicity and the clinical manifestation of drug discontinuation. This study can add to the body of work on this topic by including a large number of HIV monoinfected and HIV/HCV coinfected individuals with well characterised chronic HCV infection via HCVAb and HCV RNA testing. This allows for comparison of the rate of ARV drug discontinuation between those HCVAb negative, those that are HCVAb positive but have cleared the virus and those with on-going HCV viral replication.

A further aim of this analysis is to examine the effect of liver fibrosis on ARV drug discontinuation using the biomarker hyaluronic acid. This study can build on current knowledge by describing the relationship between on-going HCV viral replication and the presence of liver fibrosis with regards to ARV drug discontinuation. Of particular interest was to identify drug classes and individual drugs with the highest risk of discontinuation among those with viremic HCV infection and liver fibrosis, which could prove to be valuable information for clinicians when deciding which treatments to recommend in these hard to treat patients.

6.3 Methods

6.3.1 Patient selection

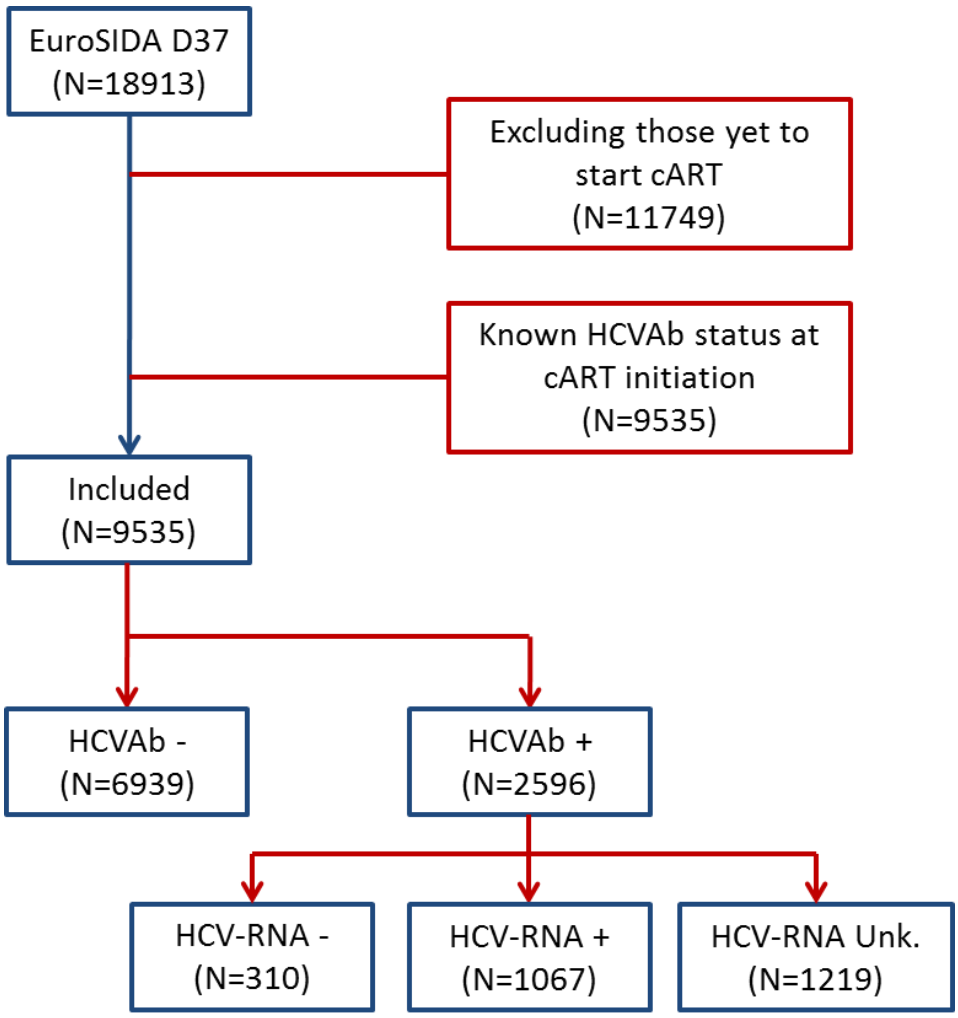
The D37 update of the EuroSIDA database included 18,913 HIV-positive individuals from 108 centres across Europe, Israel and Argentina. Patient follow-up of the current study population is to the end of December 2012. All EuroSIDA patients receiving cART, defined as at least three ARVs of any class, with known HCVAb status during prospective follow-up were eligible for inclusion in this study. Figure 6.2 shows the breakdown of how individuals were selected for inclusion in this study. Excluding individuals yet to have initiated cART there were 11,749 individuals in EuroSIDA. Of those, 9,535 had known HCVAb status at or before cART initiation during prospective follow-up. At baseline the largest proportion of individuals included were HCVAb negative (72.7%). Among HCVAb positive individuals with known HCV RNA status at baseline 77.5% were HCV RNA positive. HCVAb negative individuals are retained in this study to compare the rate of drug discontinuation among HIV-monoinfected individuals with those coinfected with HCV.

HA was previously measured in EuroSIDA among a subset of individuals that were positive for HCVAb and/or HBsAg with available stored plasma samples[622]. All individuals that developed a liver-related event during follow-up had HA measured in a baseline sample and in the last available sample prior to their event, plus at most twice between these dates if stored samples were available. A control group was also randomly selected among coinfected individuals those that did not develop a liver-related event with HA measured at similar time points. For the purpose of this study there are a maximum of four HA measurements available for each patient. As HA was measured from stored plasma samples all measurements are taken per protocol and not as a result of clinical disease management. Further, the technicians that performed the measurements were blinded as to whether a liver-related event had occurred.

6.3.2 Statistical methods

Throughout the study baseline was defined as the date of initiating a new cART regimen, defined as three ARV drugs of any class (referring either to the first cART regimen or a new regimen due to drug switching or treatment breaks), recruitment to EuroSIDA, or 1st January 1999 (when EuroSIDA began collecting reasons for treatment discontinuation), whichever occurred later. Follow-up was counted from baseline until any component of cART was discontinued. Patients were censored one month prior to the initiation of interferon-based therapy due to the potential for drug switches in order to avoid drug interactions with HCV treatment. If after a discontinuation an individual remained on three or more ARVs or subsequently initiated cART again at a later date, they re-entered the

Figure 6.2 Inclusion criteria; baseline is defined as the date of first initiating cART, recruitment to EuroSIDA or 1st January 1999, whichever occurred later



analysis with a new baseline and follow-up was counted until the next ARV drug discontinuation.

The endpoints of this study were instances of stopping an ARV drug where the reason for stopping was attributed to toxicity or patient/physician choice (TOXPC). The inclusion of a toxicity-related discontinuation is clear, but patient and physician choice could potentially be attributed to a number of underlying reasons. However, it was decided that many of the underlying causes of an ARV drug discontinuation due to patient or physician choice could be due to side effects relating to ARV toxicity and hepatotoxicity.

Switches from single agent drugs to combination pills containing the same drugs were not considered to be treatment discontinuations, for example switching a 2 pill regimen of efavirenz and Truvada to a single pill regimen of Atripla. In EuroSIDA clinical sites have

different methods of reporting ARV drug regimens and their start and stop dates. Some sites will always list the individual components of a regimen even if the regimen consists of a single combination pill, while other sites would simply list the name of the combination pill. Therefore, it is not always possible to differentiate between combinations pills and single agents. Consequently, for the purposes of this study, when more than one drug is stopped at the same time the reasons for discontinuing those drugs are attributed to all the drugs.

Multivariable Poisson regression models were used to assess the risk of ARV drug discontinuation due to TOXPC among different HCV infection profiles for drug classes and individual drugs. A separate model was used for each ARV drug class and individual ARV drug. Generalised estimating equations incorporating an unstructured covariance structure and robust standard errors were used allowing for multiple discontinuation endpoints per individual included in the study.

The analysis of this study is split into two sections, the first deals with the population of individuals with known HCVAb. In the analysis of this population the following variables are adjusted for in multivariable regression:

- Age/Sex/Race
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- HIV transmission risk group
- HCV genotype
- HCV RNA (time-updated)
- HIV RNA (time-updated)
- CD4 cell count (time-updated)
- HBsAg status (time-updated)
- Baseline calendar year

The second section of the analysis in this chapter deals with the subgroup of individuals with HA measured. Studies have shown that HA remains relatively stable over time in individuals that do not develop liver-related events, typically increasing by no more than 1ng/ml per year[622]. With this in mind it was decided to count follow-up within a 2-year window either side of available HA measurements in order to maximise the power of the study. Multivariable Poisson models were again used to assess the risk of ARV drug discontinuation due to TOXPC for drug classes and individual drugs, adjusting for the same covariates listed above plus a binary variable for HA (>100ng/ml vs. ≤100ng/ml).

To analyse as many individual ARV drugs as possible while retaining statistical power, analysis of individual ARV drugs was restricted to those in which there were at least 25 discontinuation events in the HCV RNA population and the HA population.

6.3.3 Sensitivity analyses

Two sensitivity analyses were performed. The first was to further adjust for time-updating alanine transaminase (ALT) and aspartate transaminase (AST) levels, in patients with these data available. ALT and AST levels above the normal range are by definition considered to be signs of hepatotoxicity and in many cases would indicate the need to discontinue ARV treatment[1]. Therefore, these sensitivity analyses aim to examine whether HCV RNA and HA levels are predictors of ARV drug discontinuation independent of raised liver enzymes.

Figure 6.2 shows that at baseline a large proportion of HCVAb positive individuals had unknown HCV RNA levels. During the course of follow-up the proportion of individuals with known HCV RNA increased greatly. In the second sensitivity analysis, to see if missing HCV RNA data during the early stages of follow-up were a source of bias in the results, an analysis using multiple imputations was used to replace the missing HCV RNA data. Multiple imputations were performed using the PROC MI procedure in SAS. This procedure uses the whole spectrum of available data for each individual to estimate whether individuals with unknown HCV viremia are positive or negative. The full list of variables above was used to impute the missing HCV RNA data. The data were imputed three times, generating three independent sets of complete data and then analysed together to obtain overall estimates incorporating uncertainty relating to the missing data.

6.4 Results

6.4.1 HCV RNA and ARV drug discontinuation

6.4.1.1 Generalizability and baseline characteristics

In the patient selection process for this analysis, 9,378/18,913 EuroSIDA participants were excluded as they had not yet started cART or had unknown HCVAb status. In multivariable logistic regression, compared with the whole EuroSIDA population, those with documented history of cART use and known HCVAb status were more likely to be male (aOR: 1.19 (95% C.I. 1.08 – 1.31; *P*=0.0003) vs. female) and reside in Southern or Northern Europe (aOR: 1.28 (95% C.I. 1.15 – 1.42; *P*<0.0001) and aOR: 1.15 (95% C.I. 1.03 – 1.28; *P*=0.011), respectively) but not East Central Europe (aOR: 0.64 (95% C.I. 0.56 – 0.73; *P*<0.0001)), compared with Western Europe. Those eligible for inclusion were also less likely to belong to the men who have sex with men (MSM) or heterosexual HIV transmission risk groups (aOR: 0.74 (95% C.I. 0.67 – 0.84; *P*<0.0001) and aOR: 0.84 (95% C.I. 0.75 – 0.93; *P*=0.0009)) compared with injecting drug users. The population eligible for inclusion also had more recent enrolment to EuroSIDA (aOR: 1.77 (95% C.I. 1.70 – 1.84; *P*<0.0001) per 5 years).

Baseline characteristics of this population, split by HCV status, are shown in Table 6.2. The majority of individuals were white (88.3%) men (73.3%) with a median age of 41 (IQR: 35 – 48). In general, individual demographics were similarly distributed by HCV status. Though notably, 75.1% of those positive for HCVAb with detectible HCV RNA were injecting drug users, compared with 2.9% of those HCVAb negative. Other minor differences at baseline included, a lower proportion of HCV RNA positive individuals with NNRTI treatment experience compared to HCVAb positive individuals with undetectable HCV RNA (33.7% vs. 38.7%), and a lower proportion of HCV RNA positive individuals HBsAg positive, with multiple viral hepatitis, compared to HCVAb positive individuals with undetectable HCV RNA (5.9% vs. 12.3%). Among HCVAb positive individuals with detectible HCV RNA, the HCV genotype distribution was genotype 1 (48.4%), genotype 2 (3.0%), genotype 3 (24.7%) and genotype 4 (13.4%). There were a further 10.5% that had no HCV genotype data available. The median baseline HCV viral load for the HCV RNA positive group was 5.8$\log_{10}$ IU/ml (IQR: 5.3 – 6.3).

Table 6.2 Baseline characteristics by HCV status

Median (IQR) / %		***All (N=9535)***	***HCVAb- (N=6939)***	***HCVAb+ / HCV RNA- (N=310)***	***HCVAb+ / HCV RNA+ (N=1067)***	***HCVAb+ / HCV RNA Unk. (N=1219)***	***P-value[1]***
Age		41 (35 - 48)	42 (36 - 50)	40 (35 - 45)	39 (34 - 44)	37 (33 - 41)	<0.0001
Male		73.3	75.6	63.6	68.0	67.4	<0.0001
White		88.3	86.5	90.0	91.8	95.3	<0.0001
Diabetic	No	83.7	83.2	82.6	82.6	88.0	0.0002
	Yes	3.7	4.0	5.2	2.9	2.5	
	Unknown	12.6	12.8	12.3	14.5	9.5	
Hypertensive	No	49.9	49.1	53.2	51.4	52.0	<0.0001
	Yes	18.5	20.9	14.5	12.7	11.2	
	Unknown	31.6	30.0	32.3	36.0	36.8	
Smoking status	Never	24.8	29.0	13.6	12.9	14.4	<0.0001
	Current	26.0	21.3	41.9	36.8	39.2	
	Former	3.0	2.8	4.2	3.0	3.5	
	Unknown	46.2	46.9	40.3	47.2	42.9	
ART use	NRTI	98.7	98.5	99.4	98.7	99.4	0.052
	NNRTI	41.3	42.3	38.7	33.7	43.2	<0.0001
	PI	61.6	61.4	63.9	68.7	56.0	<0.0001
	Other	4.1	4.8	3.6	2.5	1.6	<0.0001

Region	South	24.3	22.9	27.4	27.7	29.0	<0.0001
	West Central	24.7	27.7	24.2	23.9	8.4	
	North	22.7	26.1	16.5	15.8	10.7	
	East Central	13.4	12.5	21.0	19.4	11.3	
	East	11.7	7.5	10.0	11.1	37.0	
	Argentina	3.2	3.3	1.0	2.3	3.7	
Transmission group	MSM	40.9	53.4	11.0	7.2	7.0	<0.0001
	IDU	22.0	2.9	71.0	75.1	71.9	
	Heterosexual	30.1	36.3	11.9	11.0	16.5	
	Other	6.9	7.4	6.1	6.8	4.7	
HBsAg	Negative	87.4	88.7	82.3	86.3	82.6	<0.0001
	Positive	6.6	6.2	12.3	5.9	7.6	
	Unknown	6.0	5.1	5.5	7.8	9.8	
CD4 cell count	Cells/mm^3	334 (209 - 512)	354 (225 - 532)	331 (194 - 483)	306 (184 - 485)	265 (161 - 399)	<0.0001
HIV RNA	<500 copies/ml	51.6	53.4	53.1	49.5	41.2	<0.0001

ART: Antiretroviral therapy; NRTI: Nucleoside reverse transcriptase inhibitor; NNRTI: Non-nucleoside reverse transcriptase inhibitor; PI: Protease inhibitor

Baseline defined as known HCVAb status, the date of first initiating a cART regimen (at least 3 antiretrovirals of any class), recruitment to the study, or 1st January 1999 (when EuroSIDA began collecting reasons for treatment discontinuation), whichever occurred later.

[1]*P*-value from chi-square test for difference in proportions or Kruskal-Wallis tests for difference in population distributions.

In total 8,873 ARV drug discontinuations due to TOXPC were included in this population of 9,535 individuals with known HCVAb status who had initiated cART, contributing a total of 49,215 PYFU. Over the course of follow-up 2,744 individuals tested HCVAb positive and of those HCV RNA data were available for 1,904 (69.4%). Among individuals with HCV RNA data available 1,538 (80.8%) tested positive.

The overall incidence of ARV drug discontinuation due to TOXPC in the whole population was 18.0 (95% C.I. 17.7 – 18.4) per 100 PYFU. The breakdown of reasons for ARV drug discontinuation was patient and physician choice (69%) and toxicity (31%). Among toxicities the most commonly reported reasons for discontinuation were from the gastrointestinal tract (35%), other toxicities (21%), from the nervous system (20%), from the kidneys (13%), and liver-related toxicity (7%).

6.4.1.2 HCV status and ARV discontinuation

Table 6.3 shows crude TOXPC ARV discontinuation rates for each ARV drug class stratified by HCVAb/HCV RNA status. TOXPC ARV discontinuation rates were consistently higher in those positive for HCV RNA. The rate of ARV discontinuation increased from 16.6 (16.3 – 17.0) per 100 PYFU among HCVAb negative individuals to 18.5 (16.7 – 20.2) and 23.6 (22.5 – 24.7) for those HCVAb positive negative for HCV RNA and positive for HCV RNA, respectively. The rate of ARV discontinuation among those with unknown HCV RNA was similar to those positive for HCV RNA (24.1 (22.6 – 25.6)).

Table 6.3 Crude TOXPC discontinuation rates by HCV status

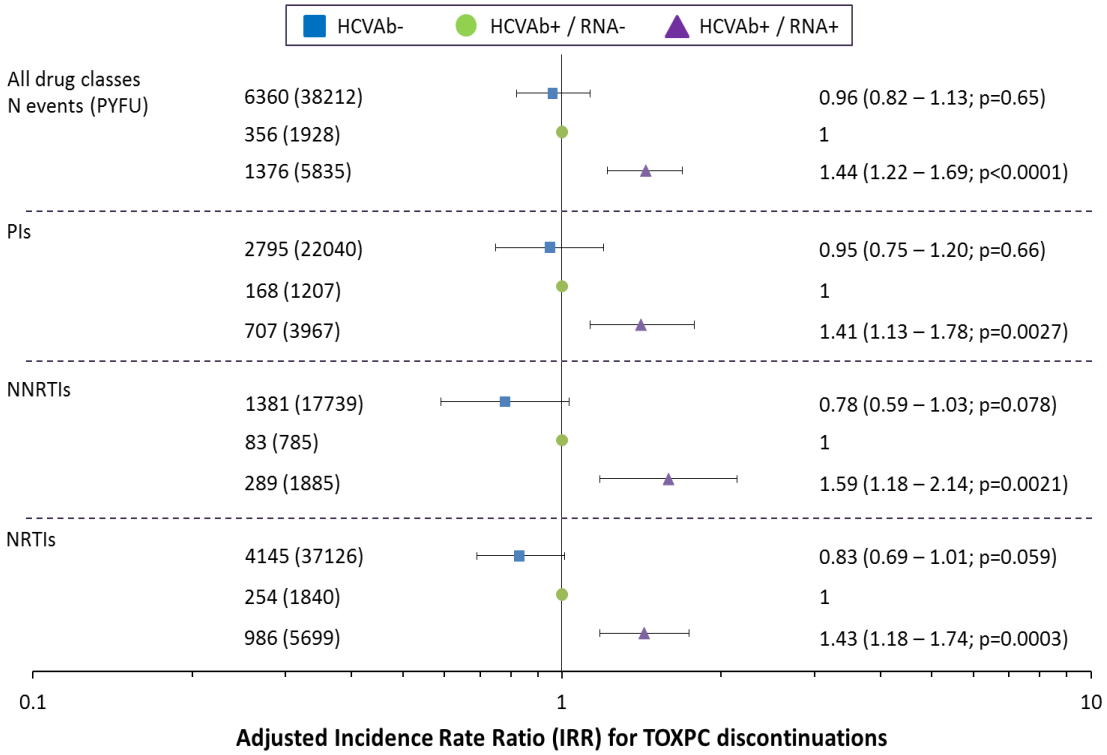
Rate /100 PYFU (95% CI)	***All ARVs***	***PIs***	***NNRTIs***	***NRTIs***
HCVAb negative	16.6 (16.3 - 17.0)	12.7 (12.2 - 13.1)	7.8 (7.4 - 8.2)	11.2 (10.8 - 11.5)
HCVAb positive / HCV RNA negative	18.5 (16.7 - 20.2)	13.9 (12.0 - 15.9)	10.6 (8.4 - 12.7)	13.8 (12.2 - 15.4)
HCVAb positive / HCV RNA positive	23.6 (22.5 - 24.7)	17.8 (16.6 - 19.0)	15.3 (13.7 - 17.0)	17.3 (16.3 - 18.3)
HCVAb positive / HCV RNA unknown	24.1 (22.6 – 25.6)	19.6 (17.8 – 21.4)	14.9 (12.9 - 16.1)	17.9 (17.3 - 18.5)

In multivariable analysis adjusted for the variables listed in the statistical methods section above, HCVAb positivity (including all HCV RNA types, positive, negative and unknown) was associated with an overall increased risk of TOXPC drug discontinuation (adjusted incidence rate ratio (aIRR): 1.28 (95% C.I. 1.15 – 1.43; *P*<0.0001) vs. HCVAb negative)

and across all ARV drug classes. Figure 6.3 shows the results after expanding this to look at the role of HCV viremia. This figure shows the adjusted rate of TOXPC drug discontinuation for those HCVAb negative and HCVAb positive with HCV viremia, compared with those HCVAb positive without HCV viremia. After adjustment, over all drug classes there was a 44% increased risk of TOXPC drug discontinuation for those with viremic HCV infection compared to those with aviremic HCV infection (aIRR: 1.44 (95% C.I. 1.22 – 1.69; *P*<0.0001)). However, there was no significant difference between the rate of drug discontinuation among those HCVAb negative and those with aviremic HCV infection (aIRR: 0.96 (95% C.I. 0.82 – 1.13; *P*=0.65)).

Similar patterns were observed within the different ARV drug class with viremic HCV infection associated with 41% (95% C.I. 1.13 – 1.78; *P*=0.0027), 59% (95% C.I. 1.18 – 2.14; *P*=0.0021) and 43% (95% C.I. 1.18 – 1.74; *P*=0.0003) increased risk of drug discontinuation compared with aviremic HCV infection, for PIs, NNRTIs and NRTIs, respectively. For NNRTIs and NRTIs, there was also borderline statistical significance

Figure 6.3 Adjusted incidence rate ratios for TOXPC drug discontinuation by HCV status

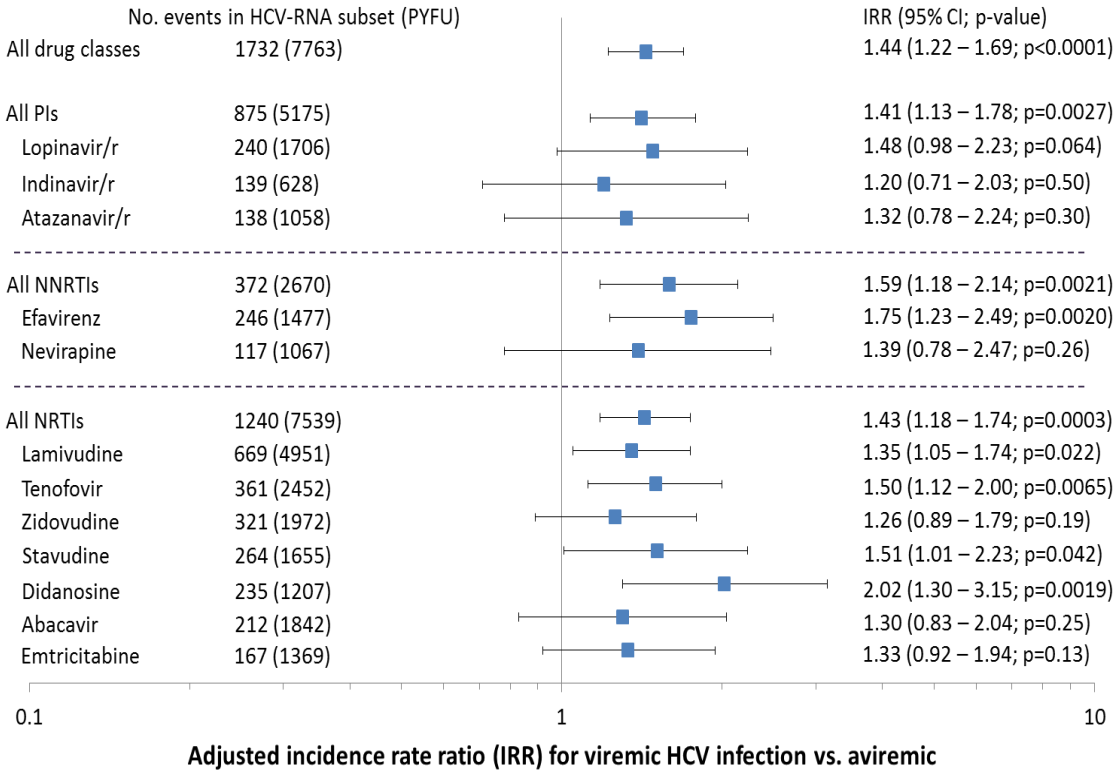


Adjusted for: age, sex, race, region of EuroSIDA, HIV transmission risk group, HCV genotype, HIV RNA, CD4 cell count, HBsAg status, and baseline calendar year

for a reduced risk of TOXPC drug discontinuation among HCVAb negative individuals compared with those with aviremic HCV infection (aIRR: 0.78 (95% C.I. 0.59 – 1.03; *P*=0.078) and aIRR: 0.83 (95% C.I. 0.69 – 1.01; *P*=0.059), respectively).

Figure 6.4 shows the difference between viremic HCV infection and aviremic HCV infection stratified by individuals ARV drugs. The number of events, PYFU and adjusted incidence rate ratios for TOXPC drug discontinuation for ARV drug classes and individual drugs are shown. For all drug classes viremic HCV infection was associated with an increased risk of ARV drug discontinuation compared with aviremic HCV infection. For all individual ARVs included in the analysis those with viremic HCV infection tended to have a higher rate of discontinuation than those with aviremic HCV infection. In particular, viremic HCV infection was significantly associated with discontinuation of the NNRTI efavirenz (aIRR: 1.75 (95% C.I. 1.23 – 2.49; *P*=0.0020)), and with the NRTIs lamivudine (aIRR: 1.35 (95% C.I. 1.05 – 1.74; *P*=0.022)), tenofovir (aIRR: 1.50 (95% C.I. 1.12 – 2.00; *P*=0.0065)), stavudine (aIRR: 1.51 (95% C.I. 1.01 – 2.23; *P*=0.042)) and didanosine (aIRR: 2.02 (95% C.I. 1.30 – 3.15; *P*=0.0019)), compared with aviremic HCV infection.

Figure 6.4 Incidence rate ratios for TOXPC drug discontinuation for viremic HCV infection versus aviremic HCV infection by ARV drug/class



Adjusted for: age, sex, race, region of EuroSIDA, HIV transmission risk group, HCV genotype, HIV RNA, CD4 cell count, HBsAg status, and baseline calendar year

6.4.1.3 ALT and AST adjustment in the HCV status population

In the set of individuals with known HCVAb status, ALT or AST was measured over the follow-up period in 8,690 of 9,535 (91.1%). The median number of measurement per individual was eight (IQR: 3 – 13) and 1,378 (14.5%) developed liver transaminase levels three times the upper limit of the normal range. Additionally adjusting for liver transaminases in the models presented in Figure 6.3, including a dummy variable for those without transaminase data, transaminase levels three times the upper limit of the normal range were consistently associated with ARV drug discontinuation for each ARV drug class. For the PI, NNRTI and NRTI ARV drug classes, liver transaminases three times the upper limit of the normal range were associated with 34% (aIRR: 1.34 (95% C.I. 1.13 – 1.59; *P*=0.0007)), 54% (aIRR: 1.54 (95% C.I. 1.22 – 1.93; *P*=0.0002)), and 25% (aIRR: 1.25 (95% C.I. 1.09 – 1.43; *P*=0.0012)) increased risk of drug discontinuation compared with liver transaminases within the normal range.

Although raised liver transaminases were clearly associated with ARV drug discontinuation, the size of the effect of viremic HCV infection compared with aviremic HCV infection remained highly significant and comparable to those shown in Figure 6.3 for all ARV drug classes. After adjustment for liver transaminases, viremic HCV infection was associated with 36% (aIRR: 1.36 (95% C.I. 1.08 – 1.71; *P*=0.0079)), 49% (aIRR: 1.49 (95% CI 1.11 – 2.01; *P*=0.0089)), and 37% (aIRR: 1.37 (95% CI 1.13 – 1.67; *P*=0.0015)) increased risk of drug discontinuation for PIs, NNRTIs and NRTIs, respectively, compared to aviremic HCV infection. Further, adjustment for liver transaminases did not alter the finding that HCVAb negative individuals were at borderline reduced risk of drug discontinuation compared with those with aviremic HCV infection among the NNRTIs and NRTIs (aIRR: 0.78 (95% C.I. 0.60 – 1.03; *P*=0.083) and aIRR: 0.84 (95% C.I. 0.70 – 1.02; *P*=0.081), respectively).

6.4.2 Hyaluronic acid and ARV drug discontinuation

6.4.2.1 Generalizability and patient characteristics

Analysis of the association between hyaluronic acid and ARV discontinuation was carried out in the subset of individuals with HA measured. HA acid has been measured from stored samples taken from patients with HCV or HBV coinfection. Therefore, the most important difference between this subset population and the main analysis is that all individuals in the subset analysis are HCVAb positive, HBsAg positive, or both.

HA was measured in 935/9535 of the main analysis population. In multivariable logistic regression, compared with the main analysis population, individuals with HA measured and

eligible for inclusion in this subset analysis were more likely to be white (aOR: 1.32 (95% C.I. 1.04 – 1.67; *P*=0.022) vs. non-white) and reside in Southern, Northern or Eastern Europe (aOR: 2.17 (95% C.I. 1.75 – 2.70; *P*<0.0001), aOR: 1.32 (95% C.I. 1.07 – 1.61; p=0.0085) and aOR: 2.21 (95% C.I. 1.43 – 3.42; *P*=0.0004), respectively), but not East Central Europe (aOR: 0.67 (95% C.I. 0.51 – 0.87; p *P*=0.0030)) compared with Western Europe.

Those included in the subset analysis were also more likely to belong to the men who have sex with men and heterosexual HIV transmission risk groups (aOR: 6.67 (95% C.I. 5.48 – 8.12; *P*<0.0001) and aOR: 7.40 (95% C.I. 5.89 – 9.30; *P*<0.0001), respectively) compared with injecting drug users. Patients included in the HA subset also had higher baseline CD4 cell counts and HIV RNA levels (aOR: 1.07 (95% C.I. 1.03 – 1.11; *P*=0.0002) per 100 cells and aOR: 1.11 (95% C.I. 1.04 – 1.17; *P*=0.0009) per $\log_{10}$ change), while they also had more recent entry to EuroSIDA (aOR: 2.04 (95% C.I. 1.83 – 2.28; *P*<0.0001) per 5 years), compared to the full main analysis population.

Plasma HA levels were measured in 935 HCVAb positive or HBsAg positive individuals. The median number of HA measurements per individual in this subset was 2 (IQR: 2 – 2; range 1 – 4). In total there were 455 ARV drug discontinuations due to TOXPC observed in 1,707 PYFU, giving an overall incidence of 26.7 (95% C.I. 24.6 – 28.8) TOXPC drug discontinuations per 100 PYFU. Of note, as this subset only includes HCVAb or HBsAg positive individuals, the overall incidence of TOXPC drug discontinuation was somewhat higher than in the main analysis (18.0 (95% C.I. 17.7 – 18.4) per 100 PYFU).

Baseline characteristics of the HA subset are shown in Table 6.4. The HA categories were generally well-balanced, though there were more individuals from the West Central region (40.1% vs. 30.5%) and fewer individuals HCVAb negative (19.7% vs. 32.4%) with HA more than 100ng/ml compared with those with HA ≤100ng/ml. However, all HCVAb negative individuals in this population subset were HBsAg positive. Those with HA more than 100ng/ml also had lower median baseline CD4 cell counts (241 (IQR 140 -390) vs. 308 (IQR 190 – 450)) compared with those with HA ≤100ng/ml.

Table 6.4 Baseline characteristics by HA status

Median (IQR) / %		***All (N=935)***	***HA ≤100ng/ml (N=788)***	***HA >100ng/ml (N=147)***	***P-value[1]***
Age		39 (34 - 44)	38 (34 - 43)	41 (38 - 47)	<0.0001
Male		75.0	74.1	79.6	0.16
White		85.8	86.2	83.6	0.45
Region	South	22.8	22.7	23.1	0.0074
	West Central	32.0	30.5	40.1	
	North	23.2	22.6	26.5	
	East Central	17.9	19.5	8.8	
	East	3.7	4.2	1.4	
	Argentina	0.4	0.5	0	
Transmission group	MSM	24.6	25.4	20.4	0.0035
	IDU	54.8	54.7	55.1	
	Heterosexual	12.7	13.3	9.5	
	Other	7.9	6.6	15.0	
HCV status	HCVAb -	30.4	32.4	19.7	0.0013
	HCVAb + / HCV RNA Negative	12.3	12.6	10.9	
	HCVAb + / HCV RNA Positive	49.5	48.4	55.8	
	HCVAb + / HCV RNA Unk.	7.8	6.7	13.6	
HBsAg	Negative	62.0	61.8	63.3	0.38
	Positive	30.1	30.7	26.5	
	Unknown	7.9	7.5	10.2	

CD4 cell count	Cells/mm^3	298 (177 - 445)	308 (190 - 450)	241 (140 - 390)	0.0005
HIV RNA	<500 copies/ml	54.9	55.1	53.7	0.76

IDU: injecting drug user; MSM: men who have sex with men; Unk: unknown; HCV: hepatitis C virus; HBsAg: hepatitis B surface antigen.

[1]*P*-value from chi-square test for difference in proportions or Kruskal-Wallis tests for difference in population distributions.

All patients in the HA subset are either HCVAb positive and/or HBsAg positive.

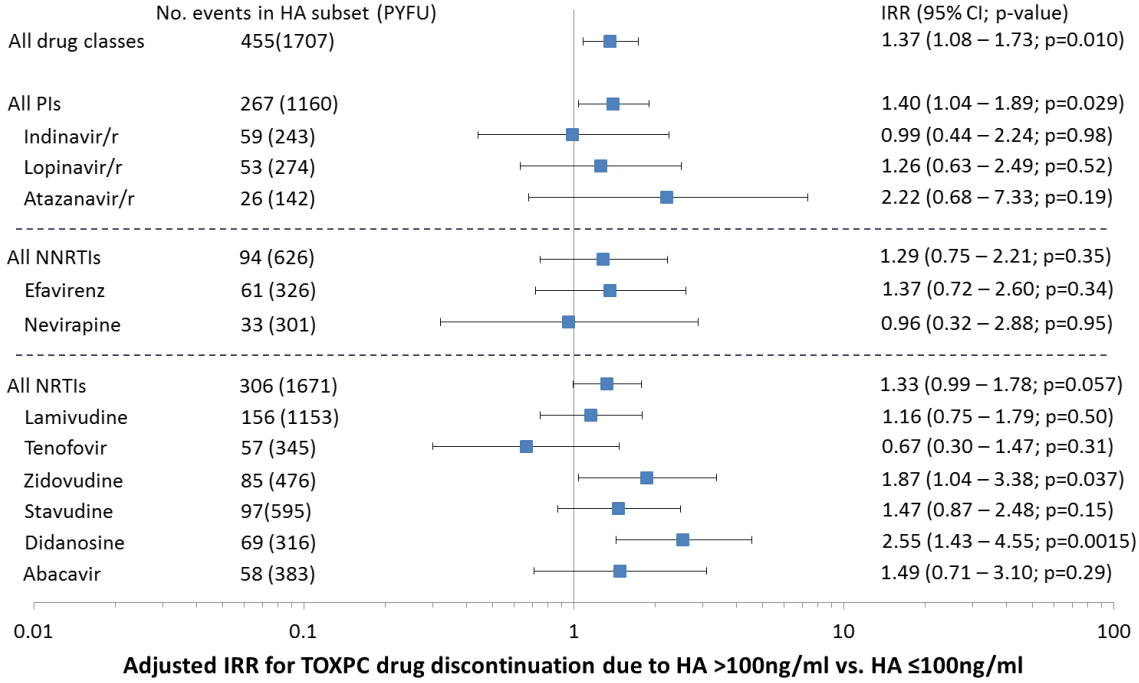
Baseline defined as known HCVAb status, the date of first initiating a cART regimen (at least 3 antiretrovirals of any class), recruitment to the study, or 1st January 1999 (when EuroSIDA began collecting reasons for treatment discontinuation), whichever occurred later.

6.4.2.2 HA status and ARV discontinuation

The crude ARV discontinuation rate was higher among those with HA >100ng/ml compared with those with HA ≤100ng/ml (35.6 (95% CI 30.0 – 41.3) and 24.9 (95% CI 22.7 - 27.2), respectively). Figure 6.5 shows adjusted incidence rate ratios for comparing HA >100ng/ml with HA ≤100ng/ml in regard to TOXPC drug discontinuation for ARV drug classes and individual drugs. After adjustment, HA greater than 100ng/ml was associated with 37% increased risk of TOXPC drug discontinuation compared with HA ≤100ng/ml (aIRR: 1.37 (95% C.I. 1.08– 1.73; *P*=0.010)) (Figure 6.5).

Interestingly in this population subset, when HA was not included as a covariate, viremic HCV infection was associated with 43% increased risk of ARV drug discontinuation (aIRR: 1.43 (95% C.I. 1.21 – 1.68; *P*<0.0001)). However, after adjustment for HA, the effect of viremic HCV infection compared with aviremic HCV infection did not approach statistical significance (aIRR of 1.00 (95% C.I. 0.66 – 1.50; *P*=0.99)). For each ARV drug class, the effect of HA greater than 100ng/ml was in the positive direction compared with HA ≤100ng/ml, reaching statistical significance among the PIs (aIRR: 1.40 (95% C.I. 1.04 – 1.89; *P*=0.029)) and borderline significance among the NRTIs (aIRR: 1.33 (95% C.I. 0.99 – 1.78; *P*=0.057)). The size of the effect among NNRTIs was comparable but did not approach statistical significance (aIRR: 1.29 (95% C.I. 0.75 – 2.21; *P*=0.35)).

Figure 6.5 Incidence rate ratios for TOXPC drug discontinuation for HA >100ng/ml versus HA ≤100ng/ml by ARV drug/class



Adjusted for: age, sex, race, region of EuroSIDA, HIV transmission risk group, HCV genotype, HIV RNA, CD4 cell count, HBsAg status, and baseline calendar year

For individual ARV drugs, the general tendency was for higher rates of TOXPC discontinuation among those with HA >100ng/ml compared with HA ≤100ng/ml. However, this effect only reached statistical significance for the NRTIs zidovudine (aIRR: 1.87 (95% C.I. 1.04 – 3.38; *P*=0.037)) and didanosine (aIRR: 2.55 (95% C.I. 1.43 – 4.55; *P*=0.0015)) (Figure 6.5).

6.4.2.3 ALT and AST adjustment in the HA status population

In the HA population subset ALT or AST was measured in 716/935 (76.6%) individuals over the follow-up period. The median number of measurements per person was 3 (IQR: 2 – 5) and 134 (14.3%) developed liver transaminase levels three times the upper normal range. Additionally adjusting for liver transaminases in the models shown in Figure 6.5, including a dummy variable for those without liver transaminase data, transaminases three times the upper normal range were consistently associated with an estimated 15% increased risk of TOXPC drug discontinuation. However, this effect did not approach statistical significance for any ARV drug class (aIRR: 1.16 (95% C.I. 0.65 – 2.07; *P*=0.62), aIRR: 1.13 (95% C.I. 0.44 – 2.93; *P*=0.80) and aIRR: 1.17 (95% C.I. 0.67 – 2.05; *P*=0.58), for PIs, NNRTIs and NRTIs, respectively). Consequently, additionally adjusting for liver transaminases did not alter the size or significance of the estimated effects shown in Figure 6.5.

6.4.3 Sensitivity analyses

6.4.3.1 Multiple imputations for missing HCV RNA data

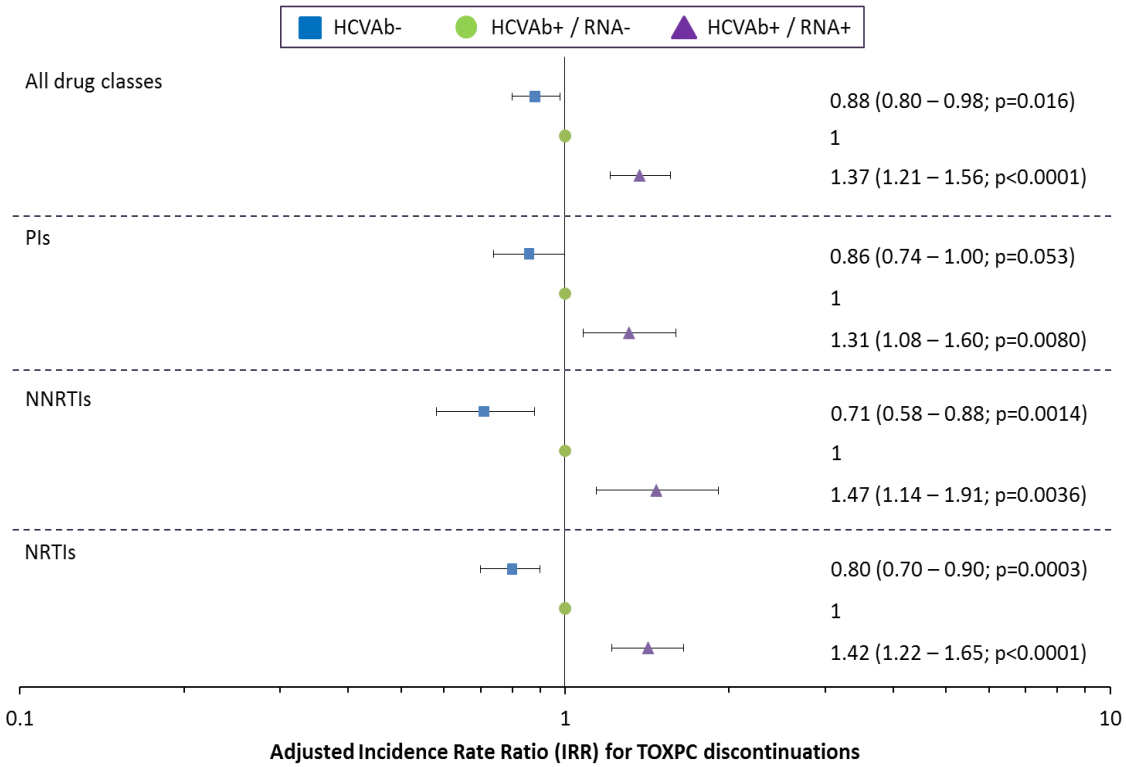
The first stage of analysis in this chapter focused on the role of HCV status, in particular the role of on-going HCV viral replication, with regards to TOXPC ARV drug discontinuations. Table 6.2 shows that at baseline 1,219 of 2,596 (47.0%) HCVAb positive individuals had unknown viremia. This could have been because HCV RNA data was not available at all clinical sites, or that HCV RNA testing had not been performed at that time point potentially far in the past. Indeed, over the course of follow-up the number of HCVAb positive individuals with known viremia status increased to 69.4%. However, there remain a large proportion of individuals with unknown viremia, which could potentially bias the results presented in Figure 6.3.

To test the impact of this potential bias a multiple imputations analysis replacing the missing HCV RNA data was performed repeating the analysis in Figure 6.3. The data were imputed three times and then analysed as a whole to take account of the uncertainty in estimating missing values. Figure 6.6 displays the results of the multiple imputations analysis and overall the results are similar to the original analysis. Overall in the imputed analysis, viremic HCV infection was associated with 37% increased risk of TOXPC drug

discontinuation compared with aviremic HCV infection (aIRR: 1.37 (95% C.I. 1.21 – 1.56; *P*<0.0001)), which is similar to the 44% increased risk shown in the main analysis. For individual drug classes, the imputed analysis estimated increased risks for viremic HCV infection of 31% (aIRR: 1.31 (95% C.I. 1.08 – 1.60; *P*=0.0080)), 47% (aIRR: 1.47 (95% C.I. 1.14 – 1.91; *P*=0.0036)), and 42% (aIRR: 1.42 (95% C.I. 1.22 – 1.65; *P*<0.0001)) for the PIs, NNRTIs and NRTIs, respectively, which is comparable to the 41%, 59% and 43% increased risks estimated in the main analysis (Figure 6.6).

In the main analysis, there was borderline significance to suggest that HCVAb negative individuals were at reduced risk of TOXPC drug discontinuations compared with those with aviremic HCV infection, for the NNRTIs and NRTIs. Interestingly, in the imputed analysis, overall there was a 12% reduced risk of drug discontinuations for HCVAb negative individuals (aIRR: 0.88 (95% C.I. 0.80 – 0.98; *P*=0.016)) compared with those with aviremic HCV infection. This reduced risk for HCVAb negative individuals was also reproduced among each ARV drug class (aIRR: 0.86 (95% 0.74 – 1.00; *P*=0.053), aIRR: 0.71 (95% C.I. 0.58 – 0.88; *P*=0.0014), and aIRR: 1.42 (95% C.I. 0.22 – 0.65; *P*<0.0001), for PIs, NNRTIs and NRTIs, respectively) (Figure 6.6).

Figure 6.6 Incidence rate ratios for TOXPC drug discontinuation by HCV status using multiple imputations to replace missing HCV RNA data



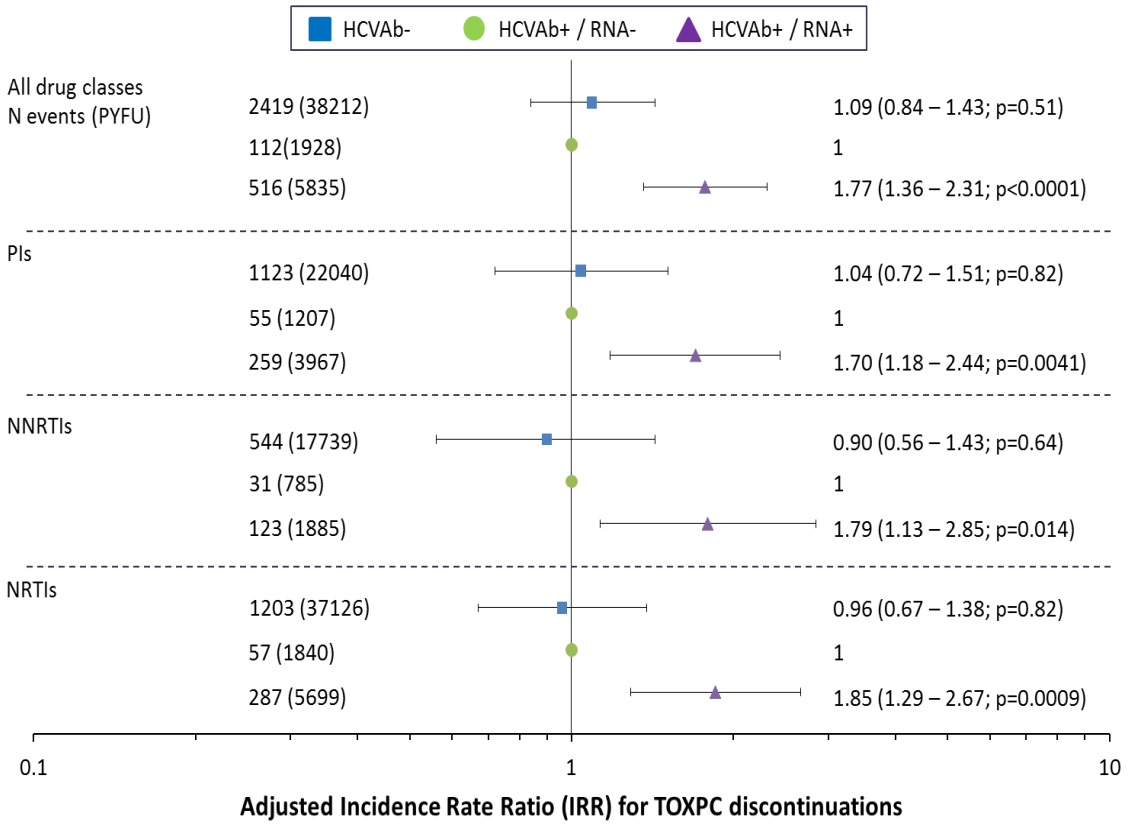
Adjusted for: age, sex, race, region of EuroSIDA, HIV transmission risk group, HCV genotype, HIV RNA, CD4 cell count, HBsAg status, and baseline calendar year

6.4.3.2 Toxicity endpoints only

So far this analysis has been based on ARV drug discontinuations due to toxicity or patient/physician choice. While definitions of toxicities are fairly clear, patient and physician choice could include a variety of different reasons for stopping ARV treatment. To see the effect that the patient and physician choice endpoints were having on the results sensitivity analysis was performed using only the toxicity endpoints. Figure 6.7 shows the main analysis repeated using toxicity endpoints only. In this analysis viremic HCV infection was associated with 77% increased risk of ARV drug discontinuation compared with aviremic HCV infection (aIRR: 1.77 (95% CI 1.36 – 2.31; *P*<0.0001)), with similar results seen for all ARV drug classes, similar to the results of the main analysis.

The main difference between this sensitivity analysis and the main analysis is that the difference between HCVAb negative individuals and those with aviremic HCV infection did not approach statistical significance overall or for any of the ARV drug classes.

Figure 6.7 Incidence rate ratios for toxicity related ARV drug discontinuations by HCV RNA status



Adjusted for: age, sex, race, region of EuroSIDA, HIV transmission risk group, HCV genotype, HIV RNA, CD4 cell count, HBsAg status, and baseline calendar year

6.4.3.3 Summary of findings

Table 6.5 summarises the findings of the analysis in this chapter. The main finding, that those with viremic HCV infection were at 35-45% increased risk of ARV drug discontinuations compared to those with aviremic HCV, was evident for all ARV drug classes. Additionally adjusting for liver transaminases, and using multiple imputations to replace the missing HCV RNA data did not alter this finding. Further, when restricting the endpoints to toxicity related discontinuation only, the effect of viremic HCV was somewhat increased, with viremic HCV patients consistently associated with >70% increased risk of ARV drug discontinuation.

In the main analysis HCVAb negativity was associated with reduced risk of TOXPC drug discontinuation for the NNRTI and NRTI ARV drug classes, with borderline statistical significance. This was also the case when additionally adjusting for liver transaminases. Further, when using multiple imputation to fill in the missing HCV RNA data, being HCVAb negative was significantly associated with a reduced risk of TOXPC drug discontinuation for all ARV drug classes. However, when restricting to toxicity endpoints only there was no difference between HCVAb negative individuals and those with aviremic HCV.

In the HA subset analysis, HA ≥100ng/ml was consistently associated with approximately 30% increased risk of TOXPC drug discontinuation for all ARV drug classes, compared with HA <100ng/ml. Further, additionally adjusting for liver transaminases did not alter this finding.

Table 6.5 Summary results and sensitivity analyses

Analysis population	***Effect***	***aIRR (95% CI)***			
		All ARVs	***PI***	***NNRTI***	***NN***
Main	HCVAb – Vs. aviremic HCV	0.96 (0.82 - 1.13; *P*=0.65)	0.95 (0.75 - 1.20; *P*=0.66)	0.78 (0.59 - 1.03; *P*=0.078)	0.83 (0.69 - 1.01; *P*=0.059)
	Viremic HCV Vs. aviremic HCV	1.44 (1.22 - 1.69; *P*<0.0001)	1.41 (1.13 - 1.78; *P*=0.0027)	1.59 (1.18 - 2.14; *P*=0.0021)	1.43 (1.18 - 1.74; *P*=0.0003)
Main: with adjustment for liver transaminases	HCVAb negative Vs. aviremic HCV	0.97 (0.83 - 1.14; *P*=0.74)	0.96 (0.76 - 1.21; *P*=0.71)	0.78 (0.60 - 1.03; *P*=0.083)	0.84 (0.70 - 1.02; *P*=0.081)
	Viremic HCV Vs. aviremic HCV	1.38 (1.17 - 1.62; *P*=0.0002)	1.36 (1.08 - 1.71; *P*=0.0079)	1.49 (1.11 - 2.01; *P*=0.0089)	1.37 (1.13 - 1.67; *P*=0.0015)
Main: with multiple imputation for missing HCV RNA data	HCVAb negative Vs. aviremic HCV	0.88 (0.80 - 0.98; *P*=0.016)	0.86 (0.74 - 1.00; *P*=0.053)	0.71 (0.85 - 0.88; *P*=0.0014)	0.80 (0.70 - 0.90; *P*=0.0003)
	Viremic HCV Vs. aviremic HCV	1.37 (1.21 - 1.56; *P*<0.0001)	1.31 (1.08 - 1.60; *P*=0.0080)	1.47 (1.14 - 1.91; *P*=0.0036)	1.42 (1.22 - 1.65; *P*<0.0001)
Main: toxicity endpoints only	HCVAb negative Vs. aviremic HCV	1.09 (0.84 - 1.43; *P*=0.51)	1.04 (0.72 - 1.51; *P*=0.82)	0.90 (0.56 - 1.43; *P*=0.64)	0.96 (0.67 - 1.38; *P*=0.82)
	Viremic HCV Vs. aviremic HCV	1.77 (1.36 - 2.31; *P*<0.0001)	1.70 (1.18 - 2.44; *P*=0.0041)	1.79 (1.13 - 2.85; *P*=0.014)	1.85 (1.29 - 2.67; *P*=0.0009)
HA subset	HA ≥100ng/ml Vs. HA <100ng/ml	1.37 (1.08 - 1.73; *P*=0.010)	1.40 (1.04 - 1.89; *P*=0.029)	1.29 (0.75 - 2.21; *P*=0.35)	1.33 (0.99 - 1.78; *P*=0.057)
HA subset: with adjustment for liver transaminases	HA ≥100ng/ml Vs. HA <100ng/ml	1.35 (1.03 - 1.77; *P*=0.032)	1.39 (0.97 - 1.98; *P*=0.072)	1.28 (0.75 - 2.20; *P*=0.36)	1.29 (0.91 - 1.83; *P*=0.15)

6.5 Discussion

Liver fibrosis is associated with ARV drug discontinuation

The analysis presented in this chapter described the incidence of ARV treatment discontinuation due to TOXPC according to HCV infection status and the level of liver fibrosis, as measured by HA. Study of this topic is of particular interest as it represents the clinical manifestation of hepatotoxicity and other toxicity caused by ARV drugs. Data from large clinical cohorts are scarce on the effect of HCV viremia and liver fibrosis on the rate of ARV drug discontinuation as they have required HCV RNA assays and in particular, liver biopsy. In this study, using the biomarker plasma HA as a surrogate for liver fibrosis, patients with HA more than 100ng/ml were found to be at increased risk of TOXPC drug discontinuations compared to those with HA less than 100ng/ml. This was true overall including all ARV drug classes and individually among the PIs, with borderline statistical significance also among the NRTIs. The effect of high HA was in the same direction, but did not reach statistical significance among the NNRTIs, which is likely due to the reduced number of events and PYFU for this drug class.

Adjustment for the liver transaminases ALT and AST did not alter these findings, which suggests that high HA, or significant liver fibrosis, is an independent predictor of ARV treatment discontinuation from raised liver enzymes. This is a particularly interesting finding for the PI drug class as although there is some degree of toxicity associated with all ARV drugs, PIs have been considered to be a more liver-friendly class of ARV[631-634]. PIs have not been strongly associated with raised liver transaminases[634], but the findings presented in this chapter could suggest that liver fibrosis can lead to PI toxicity.

Many studies have documented rapid progression of liver fibrosis in HIV/HCV coinfected individuals[376;389;395], while other studies have shown an association between liver fibrosis and ARV-related hepatotoxicity[619;635]. In one study of ARV drug induced liver injury, *Sulkowski* suggests that although the mechanism by which viral hepatitis increases the risk of drug induced hepatotoxicity is unknown, individuals with cirrhosis could have decreased P450 enzyme activity leading to increased exposure to ARVs[636]. As PIs are predominantly metabolised by the P450 enzyme system[601], the findings presented here could suggest that liver damage inhibiting P450 metabolization is causing overdosing of these ARV drugs which leads to toxicity and is manifest clinically by ARV drug discontinuation.

A plausible explanation for this finding could relate to the use of ritonavir. Except for nelfinavir, the current standard of care recommends using ritonavir as a pharmacokinetic

enhancer of PIs[1]. The basis of this recommendation is that ritonavir is known to be a potent CYP3A4 inhibitor, which acts to slow down the metabolism of PIs in the blood stream and increase exposure to these drugs[603;608;612]. It could be suggested that in individuals with high HA, or significant liver fibrosis, who may already experience decreased CYP450 activity, the inhibitory effect of ritonavir is causing the metabolism of PIs to slow down to such an extent that they are being overdosed in a normal dosage schedule. Alternatively, individuals with advanced liver fibrosis could be less tolerant to side-effects attributed to PIs, nausea, diarrhoea and insulin resistance[19].

Perhaps somewhat surprisingly, the same effect of high HA on TOXPC drug discontinuation did not reach statistical significance among NNRTIs, which are metabolised in much the same way as PIs via the P450 enzymes[601]. Potentially this could have been due to reduced statistical power in this ARV drug class, with less than half the events and PYFU observed in the other classes. Further, HA more than 100ng/ml was associated with 37% increased risk of drug discontinuation of efavirenz, though it did not reach statistical significance. In comparison, high levels of HA did not appear to be associated with discontinuation of nevirapine. Other studies to have examined the relationship between liver fibrosis and ARV-associated toxicity have acknowledged a higher risk of hepatotoxicity and raised liver transaminases during NNRTI use[637;638], specifically for nevirapine[639;640].

In this study, it is possible that HA was not found to be associated with discontinuation of nevirapine as the drug's link to liver fibrosis and tendency to raise liver transaminases is most important within the first six weeks of use[641]. In this study the median time for discontinuation of nevirapine was 7.7 months. Further, it is not possible to rule out the strong possibility of confounding by indication. The deleterious influence of nevirapine and NNRTI drug class may have been underestimated because of less frequent use of these drugs in individuals with HIV/HCV coinfection. Patients and clinicians, aware of the literature that has shown a relationship between NNRTIs, specifically nevirapine, and hepatotoxicity may have decided to avoid that drug class. Supporting evidence of this selection bias can be found in the lower number of events and PYFU for NNRTIs compared to the other drug classes.

The borderline effect of high HA seen for the NRTI drug class is driven mostly by the significant effects seen for zidovudine and didanosine, which have previously been associated with liver toxicity[642-645]. Although zidovudine and didanosine are no longer in routine use in the Western world, it remains important to study these drugs as they are still used in the resource-limited setting. These two drugs along with stavudine are contraindicated when initiating treatment for HCV[1;646;647], and ARV treatment switches away

from these drugs prior to initiating HCV treatment are likely. However, in this analysis patients were censored one month prior to initiating HCV treatment. Further, in an extra sensitivity analysis censoring patients six months prior to initiation of HCV treatment the results were identical to those presented here (data not shown).

The use of nucleoside analogues, in particular didanosine and stavudine, has been associated with increased levels of liver fibrosis among HIV-positive individuals[643;645]. It is likely that this increased liver fibrosis is leading to hepatotoxicity via reduced metabolic rates and increased exposure to ARVs. Zidovudine, although primarily removed from the body via renal excretion, has a metabolising contribution from the P450 enzyme system, and as with PIs, it may be this mechanism hindered by liver fibrosis that leads to ARV-related toxicity and treatment discontinuation[601].

HCV antibody and HCV RNA are associated with ARV drug discontinuation

HCVAb positivity was associated with an increased risk of TOXPC drug discontinuation in this study, in line with other work on the topic[536;629;630]. Expanding this to study the effect of HCV viremia on the risk of ARV drug discontinuation, individuals with viremic HCV infection were found to be at consistently higher risk of TOXPC drug discontinuation compared with those with aviremic HCV infection, with the strongest association seen among NNRTIs. Further, there was sufficient statistical power to detect significant associations between viremic HCV infection and discontinuation of the NNRTI efavirenz and NRTIs lamivudine, tenofovir, stavudine and didanosine.

HCV viral load, along with duration of HCV infection, have previously been identified as predictors of liver fibrosis[645;648]. Interestingly, as the effect of HCV viremia became non-significant when adjusting for HA in this study, these results suggest that liver fibrosis possibly caused by HCV viral replication, as measured by elevated HA, is the driving force behind ARV drug discontinuation and not viral replication *per se*.

The significant associations between viremic HCV infection and discontinuation of the NRTIs lamivudine and tenofovir can partially be explained by their use in combination pills. In EuroSIDA, due to the way data is collected at different clinical sites, it is not always possible to differentiate between combination pills and single agent regimens. In the HCV viremia analysis, there were 669 drug discontinuations of lamivudine, however, upon further examination just 25 (3.7%) of these were instances where lamivudine was the only drug stopped at that time. Further, it is also true that the effects of lamivudine and emtricitabine could have been inflated by switches from one drug to the other, as these are often considered to be equivalent treatments.

The common combination of efavirenz and tenofovir in the combination pill Atripla could explain some of the effect seen for tenofovir. Viremic HCV infection was strongly associated with discontinuation of efavirenz, those with viremia having 75% increased risk of discontinuation compared to aviremic patients. Therefore, it is possible that some of the discontinuations of Truvada were as a result of efavirenz toxicity, but these discontinuations were also attributed to tenofovir. Alternatively, viremic HCV infection has been associated with an increased risk of chronic kidney disease[26;549], which due to the primary renal excretion of tenofovir[601] and its link with chronic renal impairment[649], could often lead to discontinuation of tenofovir as a precautionary measure.

One possible explanation for the excess risk of ARV drug discontinuation among viremic HCV patients could be heightened transaminase levels among individuals with chronic HCV. These patients will have less room for treatment-induced transaminase increases before treatment will be discontinued due to hepatotoxicity. ALT and AST levels three times the upper limit of the normal range were highly significant predictors of treatment discontinuation in the HCV viremic population, which is to be expected given their indication of treatment withdrawal due to hepatotoxicity[614]. However, after adjustment for ALT and AST, the effect of viremic HCV infection overall and among all drug classes remained highly significant, indicating that HCV viremia is a significant predictor of drug discontinuation independent of raised liver transaminases.

There was borderline statistical evidence to suggest that individuals with aviremic HCV infection remained at higher risk of drug discontinuation than HCVAb negative individuals, for the NNRTI and NRTI drug classes. This finding was also given further evidence by the multiple imputations analysis, in which HCVAb negative individuals were at significantly decreased risk of drug discontinuation than aviremic HCV patients for all drug classes, although with borderline significance for the PIs. One potential explanation for the residual excess in TOXPC drug discontinuations after successful clearance of HCV viremia, in comparison to HIV monoinfected individuals, could be continuing HBV infection. In a further sensitivity analysis removing HBV positive individuals, the association between HCVAb negativity and reduced risk of drug discontinuation became non-significant among NNRTIs, but remained for the NRTIs, which suggests that confounding HBV infection can only partially explain this finding (data not shown).

It is likely that unmeasured lifestyle factors associated with HCV coinfection explain some of the differences between HCVAb negative and HCVAb positive individuals. Further, the significant differences seen between these groups in the multiple imputations analysis

could potentially be explained by the misclassification of some viremic patients to the aviremic category by the imputation method.

6.5.1 Limitations

This study has several limitations. Although HA has been identified as a promising biomarker for significant liver fibrosis, an important limitation to its use in daily clinical practise is a substantial postprandial increase in the first two hours after food intake[650]. However, the influence of food intake on this study would adversely affect both individuals that experience many drug discontinuations and those that have very few, meaning that the effect of food intake is to cloud the true relationship between HA and drug discontinuations. Therefore, rather than produce false inference, the influence of food intake would serve to underestimate the effect of HA in this study.

Due to the limited number of HA measurements available per individual in the subset analysis, a two year window either side of an HA measurement was allowed for the accrual of follow-up. This was to allow as many individuals and PYFU as scientifically feasible to be included in the subset analysis. However, the consequence of this method means it is possible that HA measurements attributed to the time of a drug discontinuation could have occurred after the event. Furthermore, in the analysis of individual drugs, especially for the HA subset analysis, the number of discontinuation events and PYFU included for each drug often meant that there was insufficient power to detect small differences in the rate of treatment discontinuation.

A combined endpoint of drug discontinuation due to toxicity or patient and clinician choice was used in this study. Whereas the definition of toxicity is clear and well-defined in EuroSIDA follow-up data collection forms, patient and clinician choice could potentially reflect many different reasons. In sensitivity analysis, the models presented here were re-run using only the toxicity discontinuations as study endpoints. This reduced the power of the analysis substantially; however, the results did not differ greater from those in the main analysis. In fact, any differences from the main analysis were to further enhance the associations between HCV viremia, HA and ARV drug discontinuation.

A further limitation of this study is that HCV RNA data were not complete. In a sensitivity analysis multiple imputations were performed in order to impute the missing data; however, this is a well-known statistical technique that produces unbiased estimates when data are missing completely at random (MCAR) or missing at random (MAR)[522]. The missing data in this analysis were HCV RNA. Section 4.4.1 of this thesis showed that missing HCV RNA data was associated with Eastern Europe. Eastern Europe is more likely to have missing

data than Western Europe due to differences in the quality of clinical management. However, this does not mean that the underlying distribution of people positive or negative for HCV RNA would be different in this region. Therefore, it is reasonable to assume these data are MAR.

It is likely that there are a number of inherent differences between HCV-positive and HCV-negative individuals. Detailed data on socio-economic factors and lifestyle may have helped to further describe patterns of ARV discontinuation among HCV-positive and HCV-negative individuals. Unfortunately, this data is not currently available in EuroSIDA and, as with all observational studies, it is not possible to exclude the influence of unmeasured confounding on the analysis.

6.5.2 Conclusions

The analyses in this chapter have documented the rate of ARV treatment discontinuation according to HCV viremia status and levels of HA. The key findings presented here are that HIV/HCV coinfected individuals with on-going viral replication were at greater risk of ARV drug discontinuation compared with coinfected individuals with aviremic infection (those who have cleared the virus), for all ARV drug classes. Interestingly, this effect seems to be explained by more advanced liver fibrosis among those with detectible HCV viremia, as HCV viremia was no longer a predictor of drug discontinuation after adjustment for HA.

The largest effect of liver fibrosis on the risk of treatment discontinuation was seen for the PI drug class, which is potentially explained by the powerful CYP450 inhibition associated with the use of ritonavir as a pharmacokinetic enhancer of PI therapy. High HA also appeared to have an effect on discontinuation of NRTIs, however, this was restricted to some of the older drugs that have been previously associated with the development of liver fibrosis and toxicity. Importantly, after adjusting for the liver transaminases ALT and AST, the effects of HCV viremia and high HA remained, suggesting they are independent risk factors for ARV treatment discontinuation from liver transaminases.

These findings have implications for the management of HIV/HCV coinfected individuals in that it may be preferable to avoid certain ARV drug classes when treating those with significant liver fibrosis. In particular, it may be advisable to avoid the use of a ritonavir boosted PI–based regimen in the presence of liver fibrosis. The combined effect of reduced CYP450 activity as a consequence of liver damage and ritonavir inhibition may lead to the overdosing of these drugs and hepatotoxicity.

Chapter 7

Liver-related death among HIV/HCV coinfected individuals, what are the implications for treatment with direct-acting antivirals?

7.1 Introduction

The substantial decline in HIV- and AIDS-related mortality, as a consequence of the introduction of highly potent combination antiretroviral therapy (cART), has seen liver-related death (LRD) assume increasing relative importance among HIV-positive individuals[372;373]. Although progression of liver disease is common with HCV infection and known to be accelerated further still in the presence of HIV coinfection[413], LRD is often associated with older age as complications of HCV-related liver disease usually take decades to develop[651]. During this period HCV coinfected individuals have many competing risks of death, such as mortality associated with injecting drug use (IDU), AIDS, cardiovascular disease, malignancies, bacterial infections, violent death and renal disease[373]. Therefore, a better understanding of the spectrum of causes of death, particularly LRD, among HIV/HCV coinfected individuals is essential so that new therapies for HCV can be channelled to those who need them most.

7.1.1 Causes of death among HIV/HCV coinfected individuals

The next decade promises to be a crucial period in the treatment of people with HCV. The development of new direct-acting antivirals (DAA) for treatment of HCV means there is now a considerable amount of optimism in the field of HCV research[446;652]. However, it will be important to ensure these treatments are applied appropriately. Causes of death among those with HCV vary according to a number of factors including the duration of chronic disease, access to effective treatments, age distribution and competing risks in the population[652]. Therefore, causes of death among those with HCV tend to fall within three categories, drug-related death, including overdose and suicide which can be common among injecting drug users (IDU), LRD, including death as a consequence of liver cirrhosis and hepatocellular carcinoma (HCC), and HIV-related death, such as AIDS-related mortality[652].

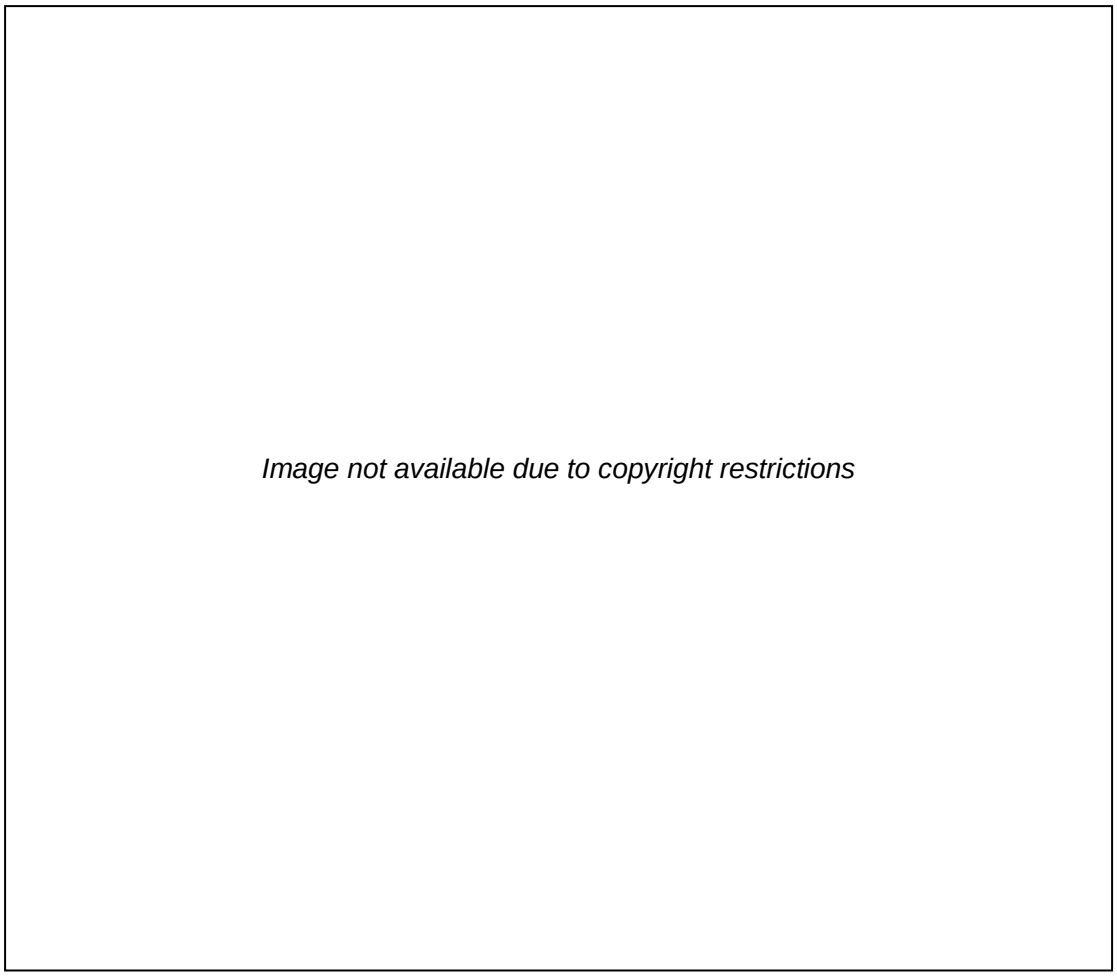
As discussed in the introduction to this thesis (Sections 1.2.5 and 1.2.6), coinfection with HIV has a negative effect on all aspects of the course of HCV infection. HIV/HCV coinfected individuals have a lower chance of spontaneous clearance of the HCV virus, higher HCV RNA levels, faster progression of liver fibrosis and lower response rates to interferon-based treatments for HCV[390;436;653-655]. In addition, although the life expectancy of HIV-positive individuals has increased dramatically as a result of modern combination antiretroviral therapy (cART), the life expectancy of HIV/HCV coinfected individuals still lags behind[655]. A recent Danish study has compared mortality rates of HIV-positive individuals and the general population. This study found that although mortality among the HIV-positive community had fallen to 19 per 1,000 person years follow-up (PYFU) after 2005 and the introduction of contemporary cART regimens, it was three-fold higher among the HIV/HCV coinfected population in the same time period (57 per 1,000 PYFU)[656].

Causes of death among HIV-positive individuals have been well-studied in recent years. In 2006, from a large study of 23,441 HIV-positive individuals, *Weber et al* reported from the D:A:D study that more than half of all deaths among HIV-positive individuals were from causes other than those associated with AIDS. Further, death from causes associated with HCV- and HBV-coinfection accounted for an increasing proportion of overall mortality[564]. LRD accounted for 14.5% of all deaths recorded in the study with the majority of these deaths occurring in those with active HCV coinfection (66.1%), active HBV infection (16.9%) or both (7.1%)[564].

Interestingly, after adjustment the authors described a strong relationship between immunodeficiency and LRD. While there were similar numbers of deaths for those with CD4 cell counts <200 cells/mm^3 and ≥200 cells/mm^3 (87 and 94, respectively), the adjusted rate of LRD in those with low CD4 cell counts was far higher (0.92 (95% CI 0.73 – 1.12) compared with 0.14 (0.11 – 0.17) per 100 person years follow-up, respectively)[564]. Further, they were able to show a clear dose-response relationship between CD4 cell count and AIDS-related death or LRD (Figure 7.1), while also reporting associations between older age, injecting drug use, HCV or HBV positivity and LRD[564].

In 2014 the D:A:D study reported an update on the underlying causes of death in people with HIV. In this large scale analysis, including nearly 50,000 participants and 4,000 deaths over the years 1999 to 2011, LRD accounted for 13% of all deaths. LRD was the third most frequent cause of death behind AIDS-related death (29%) and non-AIDS cancer (15%), but ahead of cardiovascular disease (11%)[373]. Although LRD remained one of the most important causes of death over the study period they noted a substantial decline in the

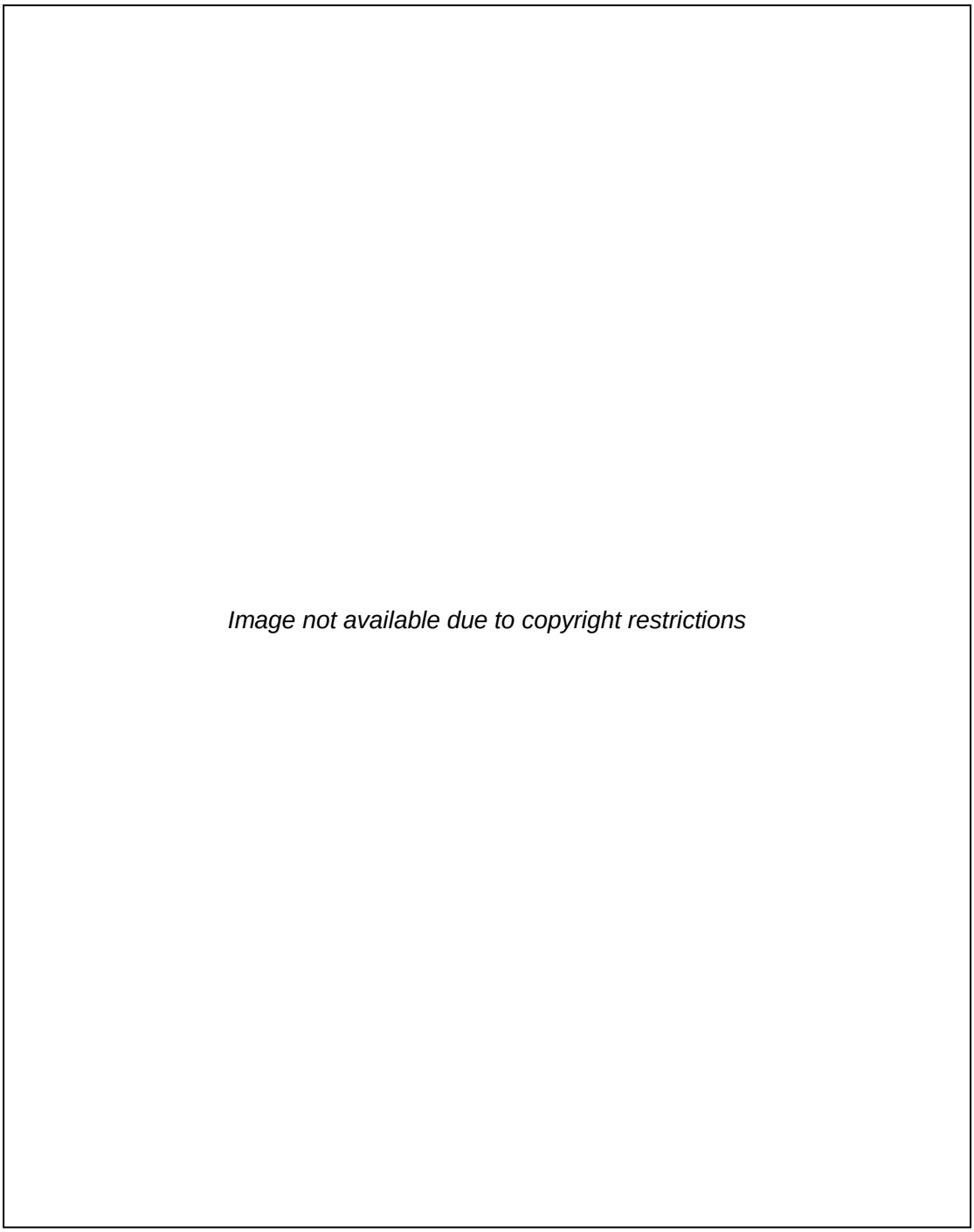
Figure 7.1 Factors associated with AIDS- and liver-related death from the DAD study, 2006[564]



number of deaths attributable to liver-related causes, decreasing by 50% from 1999-2000 to 2009-2011 (Figure 7.2)[373]. The authors propose that the fall in the incidence of death from all causes from 1999-2000 to 2009-2011 can be attributed to improvements in CD4 cell count and the introduction of less toxic ARV drugs. However, the authors also point out that the number of LRDs in those without either HBV or HCV coinfection was minimal, accounting for less than 5% of all LRDs, and that the reduction in LRD was potentially attributable to a decrease in the percentage of coinfected individuals included in the study over time[373].

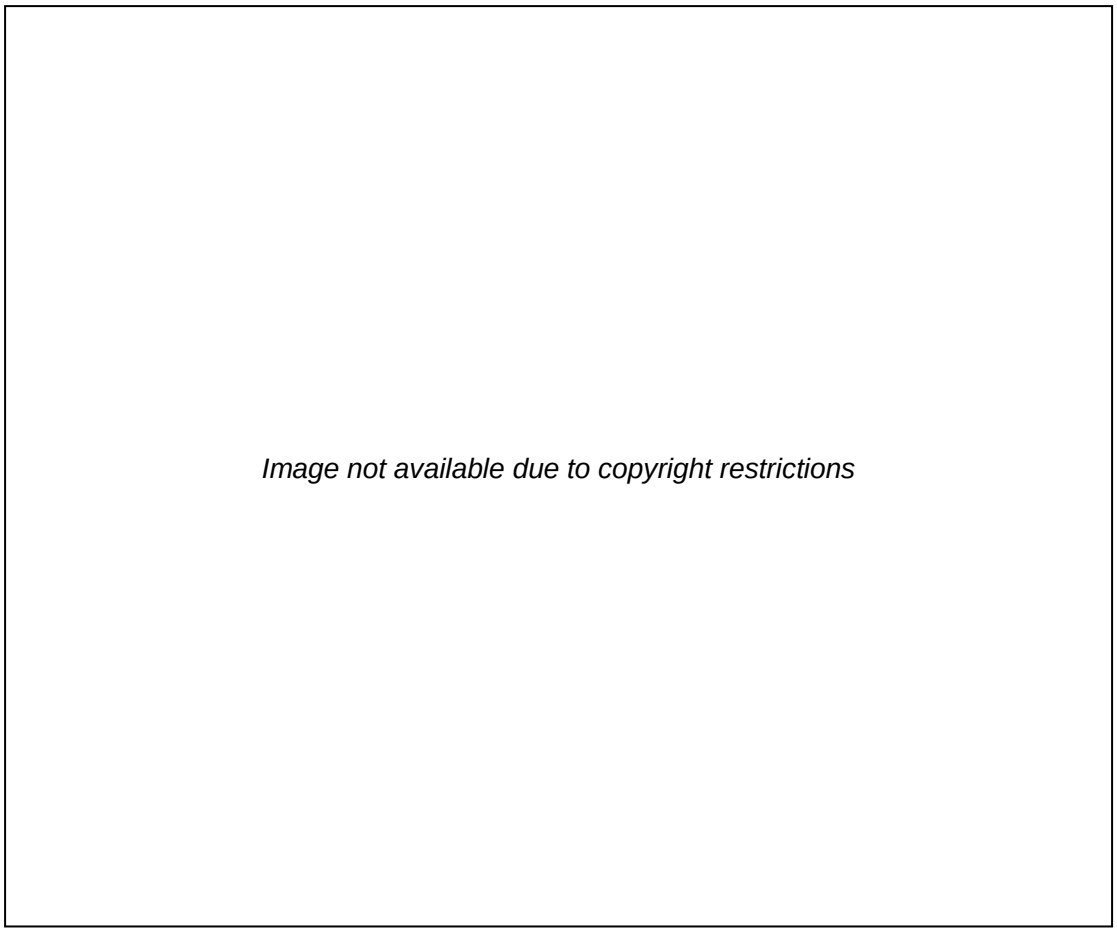
Another factor contributing to the reduction in LRD over time is thought to be the increased use of ARV drugs with activity against HBV[373]. Lamivudine, emtricitabine and tenofovir are all potent inhibitors of HBV DNA and at least 2 are now included in most ARV regimens, including combination pills such as Atripla and Eviplera which both contain emtricitabine and tenofovir[416]. Interestingly, the authors go on to say that treatment for HCV was uncommon during the course of the study and was unlikely to have significantly affected

Figure 7.2 Declining age-standardised incidence rates for specific causes of death 1999-2011[373]



the rate of LRD[373]. The French national survey also reported on causes of death among HIV-positive individuals in 2014[372]. In a large cross-sectional study looking at trends in causes of death in the years 2000, 2005 and 2010 the authors followed individuals from 90 clinical centres representing approximately 82,000 HIV-positive people in France. A total of 728 deaths were reported in 2010 with AIDS (25%), non-AIDS-non-hepatitis-related malignancies (22%) and LRD (11%) the most frequent causes of death[372]. During all three

Figure 7.3 Distribution of the underlying cause of death among HIV-positive individuals in the French national survey[372]



editions of the survey LRD has consistently been the third most frequent cause of death among those with HIV (Figure 7.3)[372].

A total of 77 deaths due to liver-related causes were reported in 2010, with 32 (41.6%) due to hepatocellular carcinoma (HCC) and 42 (54.5%) due to cirrhosis. Importantly, the authors report that 92% of the LRDs in the study were as a consequence of HBV- or HCV-coinfection[372]. Further, when analysing the coinfected individuals separately LRD became the most frequent cause of death (24%), followed by non-AIDS-non-hepatitis-related malignancies (21%) and AIDS (13%)[372]. Among those with HIV-monoinfection hepatic diseases accounted for just 1% of deaths[372].

7.1.2 Can new HCV treatments lower liver-related death rates?

The uptake of treatment for HCV remains low among HIV/HCV coinfected individuals in Europe with only approximately 25% being exposed to therapy by 2010[657]. However, in Chapter 5 of this thesis it was shown that the low uptake of treatment can at least be partially explained by the prohibitive costs of treatment, potential contraindication to

interferon-based treatments, anticipated poor treatment adherence and low treatment efficacy of pegylated-interferon plus ribavirin[657].

However, with the recent approval of less toxic, oral direct-acting antivirals (DAAs) for HCV fewer coinfected individuals will have contraindications to HCV treatment[446;658]. Further, as was discussed in detail in Section 2.2.6 of Chapter 2 of this thesis, treatment outcomes will be significantly improved with DAA therapy in comparison with interferon-based treatments. Treatment of coinfected individuals with pegylated-interferon plus ribavirin is successful for approximately 17-32% of those with HCV genotypes 1 and 4, and between 44-73% for genotypes 2 and 3[434;435;448;459], whereas gold standard DAA therapy has been shown to be successful in >90% of individuals regardless of HCV genotype or HIV-coinfection[446;465;658].

The potential benefits of curative treatment with DAA therapy are numerous, including reductions in liver fibrosis levels and therefore reductions in LRD rates and extrahepatic manifestations, as well as helping to reduce on-going transmission of the virus[659]. However, regardless of the advances made in HCV drug development, the approximate costs of treatment with these new therapies approaches €90,000 per patient with the expectation that all oral combination regimens will be more expensive still[2]. These costs will be difficult to meet in most countries and prioritisation of individuals in the greatest need of treatment will be essential.

7.2 Aims

The initial aim of this chapter was to describe causes of death among HIV/HCV coinfected individuals in EuroSIDA, paying attention to how the rate of LRD has changed over time. As AIDS-related mortality has declined in the era of highly effective cART for treatment of HIV, focus has shifted towards comorbidities among HIV-positive individuals. Therefore, a better understanding of the spectrum of causes of death among HIV/HCV coinfected individuals is essential in determining who to prioritise for expensive new treatments for HCV infection. Hence, a further aim of this chapter was to describe specific factors associated with progression to LRD, so that those at the highest risk may be prioritised for new DAA therapy. This study can add to the body of work on these topics by including a large number of coinfected individuals with well documented causes of death over a long period of follow-up.

7.3 Methods

7.3.1 Patient selection

The D38 update of the EuroSIDA database included 18,786 HIV-positive individuals from 107 centres across Europe, Israel and Argentina. Figure 7.4 shows the breakdown of how individuals were selected for inclusion in this study. All EuroSIDA individuals under prospective follow-up with documented HIV/HCV coinfection, as evidenced by a positive HCVAb test, and follow-up available after the 1st January 2000 were eligible for inclusion in this study. The D38 EuroSIDA database included 16,205 individuals with known HCVAb status, of whom 4,826 were positive and 3,941 had follow-up data recorded after 1st January 2000.

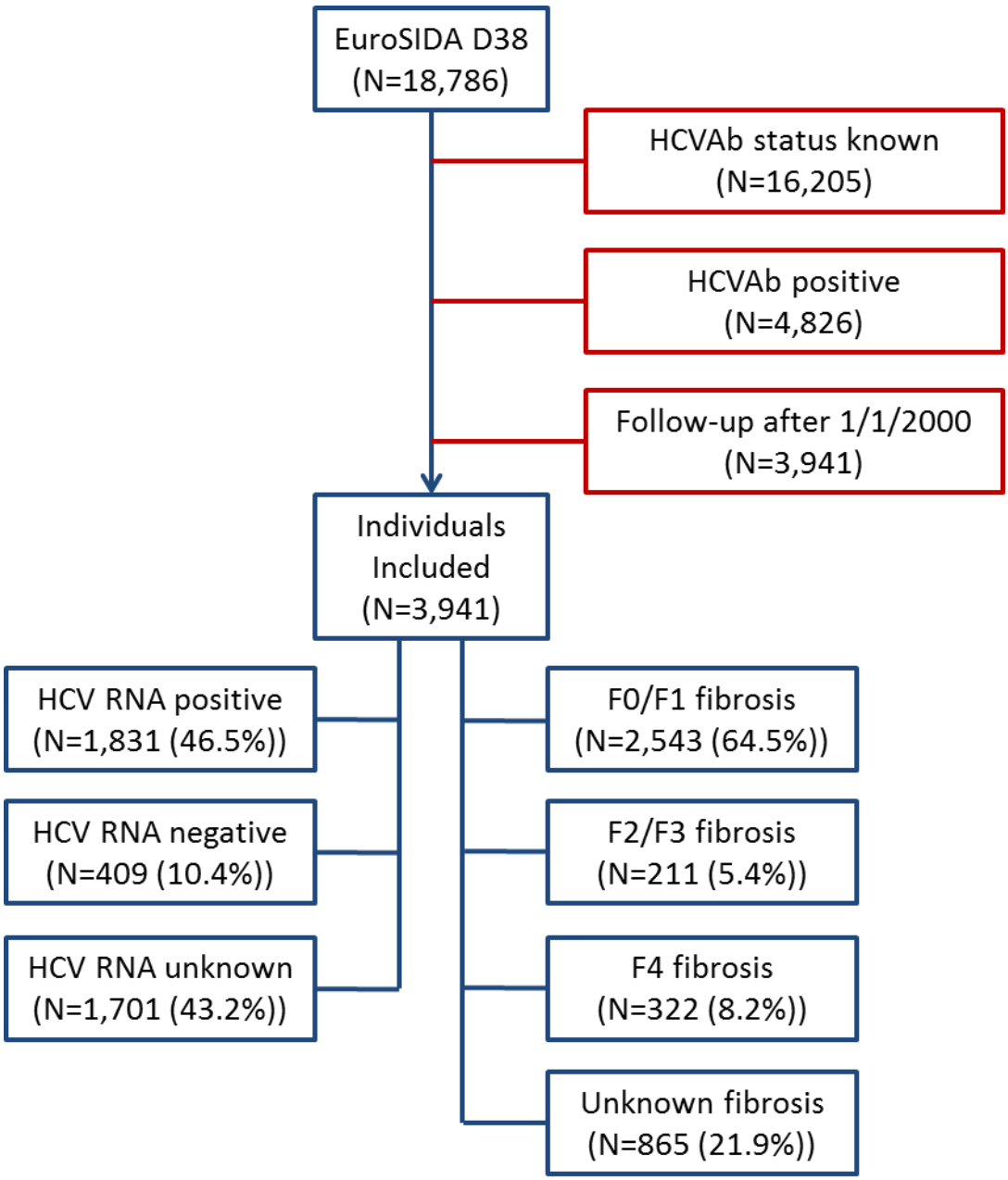
Follow-up prior to 1st January 2000 was excluded from this study as I wanted the study population to reflect the current state of clinical management of coinfected individuals. Follow-up prior to 1st January 2000 would likely include individuals receiving what today would be considered sub-optimal antiretroviral therapy. As one of the aims of this study was to provide guidance on who to prioritise for treatment with new DAA drugs for HCV, follow-up was also censored at the date of starting interferon-based treatment for HCV.

7.3.2 Statistical methods

Causes of death in this analysis were classified using the CoDe methodology described in the methodology chapter of this thesis. For the purpose of analysis deaths were classified into the following categories: LRD, AIDS-related death, non-LRD/non-AIDS-related death and unknown causes of death. Non-LRD/non-AIDS-related death includes causes of death such as bacterial infections, cardiovascular events, drug and accidental death, and cancer. However, hepatocellular carcinoma is included in the LRD category along with liver failure, cirrhosis or complications as a result of HCV or HBV infection.

Throughout this analysis baseline was defined as 1st January 2000, entry into EuroSIDA if this was after 1st January 2000, or the first fibrosis measurement available after HCVAb positivity if this occurred after entry into EuroSIDA. Crude death rates were calculated per 1000 person years follow-up (PYFU), with follow-up counted till the last study visit, death or initiation of interferon-based treatment. Crude death rates were calculated using time-updated variables so that an individual negative for HCV RNA contributed PYFU to the HCV RNA negative category until they became HCV RNA positive, at which point they contributed PYFU to the HCV RNA positive category. Crude death rates were stratified by:

Figure 7.4 Analysis population inclusion criteria and baseline HCV RNA and liver fibrosis status



- HIV transmission risk group
- Age
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- HCV viremia status (positive, negative, unknown)
- Hepatitis B surface antigen status (HBsAg) (positive, negative, unknown)
- CD4 cell count
- HIV RNA

- Reported alcohol abuse (none, current, unknown)
- Liver fibrosis staging (estimated Metavir stages F0/F1, F2/F3, F4 and unknown)

Qualitative clinician-reported data on alcohol abuse was added to the EuroSIDA data collection form in 2010. Alcohol abuse is defined as >25 units consumed per week for males and >20 units consumed per week for females. Based on these criteria the acting clinician records whether the individual is an alcohol abuser or not. Unfortunately, the number of units consumed each week is not collected, partially due to the difficulty in accurately collecting this data in the clinical setting.

A combined definition of the liver fibrosis staging was used throughout the analysis presented in this chapter according to the Metavir scoring system[420] (see Chapter 2 Section 2.2.5.3 for a description of liver fibrosis staging). F0/F1 fibrosis was defined from validated liver biopsy, Fibroscan® measurements <7.6Kpa, an APRI score <1.5 and hyaluronic acid (HA) measurements <100ng/ml. F4 fibrosis was defined from validated liver biopsy, Fibroscan® measurements >12.5Kpa, and APRI score >2 and HA measurements >250ng/ml, in line with previous EuroSIDA work and other studies of HCV infection[660-662]. Where more than one fibrosis measurement is available for each individual at each time point they are prioritised in the order given above. For example, if an individual had liver biopsy and Fibroscan® data at the same time point the liver biopsy would take precedence.

Univariable Poisson regression was used to model changes in the overall incidence of LRD over time. An interaction term comparing Western Europe and Argentina with Eastern Europe was tested to determine whether the incidence of LRD over time differed by region of EuroSIDA. Separate Poisson models were then used to describe changes in the incidence of LRD in both of these regions over time. Time-updated CD4 cell count and liver fibrosis staging variables were then added in turn to these models to assess the impact they have had on changes in the incidence of LRD.

Cox proportional hazards regression, adopting the Fine and Gray methodology of competing risks, was used to describe factors associated with progression to LRD[519]. The competing risks accounted for were death from causes other than LRD and loss to follow-up, defined as no contact with a EuroSIDA clinical site in the year prior to the median date of last follow-up for the entire EuroSIDA cohort. Multiple imputations, with four sets of replication, were used to impute missing data on HCV RNA, HCV genotype, HBsAg status and fibrosis staging, only those who were positive for HCV RNA were then included in the analysis. The following baseline covariates were included in the model:

- Age
- Gender
- Race
- Calendar year
- HIV transmission risk group
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- Previous cardiovascular events (stroke, myocardial infarction, angina, endarterectomy, high blood pressure)
- Previous diabetes diagnosis
- HCV genotype
- CD4 cell count
- Nadir CD4 cell count
- HIV RNA
- HBsAg status (positive, negative)
- Minimum duration of HCV infection
- Liver fibrosis staging (estimated Metavir stages F0/F1, F2/F3 or F4)

Minimum duration of HCV infection is the length of time prior to baseline that each individual had their first documented positive HCV antibody (HCVAb) or HCV RNA test result. Interaction terms between liver fibrosis staging, CD4 cell count and age were also tested to see if the effect of liver fibrosis on LRD was modified by CD4 cell count or age.

For comparison, a similar Cox Proportional hazards model using the Fine and Gray methodology of competing risks was used to describe factors associated with AIDS and non-AIDS/non-LRD death. The competing risks accounted for in this analysis were death from causes other than AIDS and non-AIDS/non-LRD and loss to follow-up, defined in the same way as above. The baseline covariates listed above were also adjusted for in this model.

Non-parametric cumulative incidence functions, which are not biased by the presence of competing risks, were then calculated to estimate the 5-year probability of LRD according to liver fibrosis staging and CD4 cell count.

Fine and Gray models and cumulative incidence functions were estimated using the %PSHREG and %CIF validated SAS macros[663;664].

7.4 Results

7.4.1 Generalizability and baseline characteristics

During the population selection process for this analysis 885/4,826 HCVAb positive individuals were excluded from the study as they did not have follow-up available after 1st January 2000. A total of 3,941 coinfected individuals were included in the analysis (see Figure 7.4). Multivariable logistic regression, adjusted for the covariates listed above, was used to consider how those included in the analysis differed from those that were excluded.

Those without follow-up after 1st January 2000 were less likely to reside in Western Europe (adjusted odds ratio (aOR): 0.70 (95% CI 0.52 – 0.95; *P*=0.0041)) and more likely to reside in Eastern Europe (aOR: 2.03 (1.38 – 2.99; *P*<0.0001)), compared with Southern Europe. They also had lower CD4 cell counts (aOR: 0.77 (0.69 – 0.86; *P*<0.0001) per doubling), higher CD4 cell count nadirs (aOR: 1.16 (1.07 – 1.26; *P*=0.0005) per doubling) and were far more likely to have unknown fibrosis staging (aOR: 82.4 (57.5 – 118.2; *P*<0.0001)) at last follow-up, compared to those included in the analysis.

A total of 670 deaths were recorded in the study population of 3,941 HIV/HCV coinfected individuals contributing a total of 16,091 person years of follow-up (PYFU) (median 3.5 years per person (inter-quartile range (IQR) 1.3 – 6.4)) to January 2013. The overall incidence of all-cause mortality was 41.6 (95% CI 38.6 – 44.7) per 1000 PYFU. 145/670 (21.6%) of all deaths were classified as liver-related giving an overall incidence of LRD of 9.0 (7.6 – 10.5) per 1000 PYFU.

Baseline characteristics of the 3,941 coinfected individuals included in the analysis are shown in Table 7.1 stratified by cause of death. The study population was mostly white (93.6%), males (67.9%) with a median age of 37 years. The majority of the study population resided in either Eastern (31.0%) or Southern Europe (25.5%), although all European regions were well represented. By far the most common route of HIV transmission was via injecting drug use (IDU) (70.0%) followed by heterosexual exposure (15.3%). The most frequent HCV genotype was G1 (24.3%) followed by G3 (14.2%), G4 (6.8%) and G2 (1.3%), while 53.4% had no data on HCV genotype at baseline.

Table 7.1 Baseline Characteristics of all HIV/HCV coinfected individuals included in the analysis stratified by cause of death

			Causes of death				
Median (IQR) %		***All (N=3941)***	***LRD (N=145)***	***AIDS (N=162)***	***Non-LRD non-AIDS (N=233)***	***Unknown causes (N=130)***	***P-Value****
Age		37 (31 - 43)	39 (35 - 43)	36 (31 - 42)	39 (34 - 46)	40 (35 - 46)	0.0004
Baseline date		OCT2005 (JUL2002 - JUL2008)	DEC2001 (JAN2000 - AUG2005)	JAN2005 (FEB2001 - JAN2008)	MAR2003 (JAN2000 - AUG2005)	JUN2004 (JAN2000 - JUL2006)	<.0001
Male		2677 (67.9)	103 (71.0)	117 (72.2)	180 (77.3)	95 (73.1)	0.52
White		3687 (93.6)	136 (93.8)	158 (97.5)	221 (94.8)	112 (86.2)	0.0008
Region of EuroSIDA	South	1005 (25.5)	42 (29.0)	29 (17.9)	46 (19.7)	17 (13.1)	<.0001
	West Central	551 (14.0)	28 (19.3)	12 (7.4)	25 (10.7)	34 (26.2)	
	North	495 (12.6)	37 (25.5)	24 (14.8)	74 (31.8)	34 (26.2)	
	East Central	562 (14.3)	13 (9.0)	10 (6.2)	29 (12.4)	12 (9.2)	
	East	1223 (31.0)	23 (15.9)	80 (49.4)	54 (23.2)	31 (23.8)	
	Argentina	105 (2.7)	2 (1.4)	7 (4.3)	5 (2.1)	2 (1.5)	
HIV transmission route	MSM	344 (8.7)	8 (5.5)	9 (5.6)	17 (7.3)	7 (5.4)	0.41
	IDU	2760 (70.0)	113 (77.9)	122 (75.3)	188 (80.7)	106 (81.5)	
	Heterosexual	603 (15.3)	12 (8.3)	23 (14.2)	19 (8.2)	11 (8.5)	
	Other	234 (5.9)	12 (8.3)	8 (4.9)	9 (3.9)	6 (4.6)	
HCV-RNA status	Negative	409 (10.4)	10 (6.9)	13 (8.0)	32 (13.7)	15 (11.5)	<.0001
	Positive	1831 (46.5)	87 (60.0)	55 (34.0)	120 (51.5)	67 (51.5)	

			Causes of death				
Median (IQR) %		***All (N=3941)***	***LRD (N=145)***	***AIDS (N=162)***	***Non-LRD non-AIDS (N=233)***	***Unknown causes (N=130)***	***P-Value****
	Unknown	1701 (43.2)	48 (33.1)	94 (58.0)	81 (34.8)	48 (36.9)	
Minimum duration of HCV infection	Years	2.8 (0.7 - 6.0)	4.1 (2.2 - 7.0)	2.1 (0.2 - 5.2)	4.0 (1.5 - 6.7)	3.9 (0.8 - 7.5)	<.0001
HCV genotype	G1	959 (24.3)	47 (32.4)	25 (15.4)	52 (22.3)	34 (26.2)	0.0020
	G2	52 (1.3)	2 (1.4)	4 (2.5)	3 (1.3)	4 (3.1)	
	G3	558 (14.2)	22 (15.2)	16 (9.9)	45 (19.3)	20 (15.4)	
	G4	268 (6.8)	8 (5.5)	4 (2.5)	9 (3.9)	9 (6.9)	
	Unknown	2104 (53.4)	66 (45.5)	113 (69.8)	124 (53.2)	63 (48.5)	
HBsAg status	Negative	3338 (84.7)	116 (80.0)	136 (84.0)	202 (86.7)	104 (80.0)	0.0021
	Positive	281 (7.1)	23 (15.9)	17 (10.5)	18 (7.7)	8 (6.2)	
	Unknown	322 (8.2)	6 (4.1)	9 (5.6)	13 (5.6)	18 (13.8)	
CD4 cell count	Cell/mm^3	382 (239 - 563)	216 (100 - 393)	200 (76 - 357)	303 (153 - 515)	277 (140 - 485)	<.0001
CD4 nadir	Cell/mm^3	166 (72 - 288)	96 (36 - 200)	101 (28 - 205)	115 (52 - 220)	113 (50 - 211)	0.29
HIV RNA	<400copies/ml	2048 (59.0)	60 (44.8)	27 (24.1)	105 (49.1)	51 (45.5)	0.0002
Liver fibrosis	F0/F1	2543 (64.5)	33 (22.8)	66 (40.7)	116 (49.8)	52 (40.0)	<.0001
	F2/F3	211 (5.4)	17 (11.7)	7 (4.3)	11 (4.7)	10 (7.7)	
	F4	322 (8.2)	38 (26.2)	13 (8.0)	19 (8.2)	15 (11.5)	
	Unknown	865 (21.9)	57 (39.3)	76 (46.9)	87 (37.3)	53 (40.8)	

***P-value from Kruskal-Wallis or Chi-square test comparing the individual causes of death**

LRD: Liver-related death; MSM: Men who have sex with men; IDU: Injecting drug users; HBsAg: Hepatitis B surface antigen

Alcohol abuse is omitted from this table as it was added to the EuroSIDA CRF in 2010. During follow-up, 60.5% of the study population have information on alcohol abuse with 13.4% reporting current alcohol abuse.

Baseline defined as 1st January 2000, entry into EuroSIDA if this was after 1st January 2000, or the first fibrosis measurement available after HCVAb positivity if this occurred after entry into EuroSIDA.

Interestingly, Eastern Europe, as the largest contributor of participants to the study, accounted for 49.4% of all AIDS-related deaths but only 15.9% of LRDs. In comparison, Southern Europe, the second largest contributor of participants in the study, accounted for far fewer AIDS-related deaths (17.9%) and more LRDs (29.0%) (P<0.0001). Further, 60% of those who died of LRD were HCV RNA positive compared with 34.0% of those who died of AIDS (P<0.0001), although a large proportion of those who died of AIDS had unknown HCV RNA (58.0%). A higher proportion of those who died of LRD were also hepatitis B surface antigen (HBsAg) positive (15.9%) compared with those who died of AIDS (10.5%) (P=0.0021).

At baseline 78.1% of the coinfected individuals included in the analysis had available data on liver fibrosis. Overall, 64.5% had F0/F1 fibrosis, 5.4% F2/F3 fibrosis and 8.2% F4 fibrosis. Among those who died of LRD, 26.2% had F4 fibrosis at baseline and 22.8% had F0/F1. In comparison, of those who died of AIDS just 8.0% had F4 fibrosis at baseline and 40.7% had F0/F1 (P<0.0001).

7.4.2 Crude death rates

The most common causes of death recorded in this study were AIDS (24.2%) and LRD (21.6%), followed by unknown causes (19.4%), drug/violent death (9.6%), bacterial infection (7.9%), cardiovascular disease (6.9%) and cancer (4.5%). Crude death rates (cDR) for LRD, AIDS, non-LRD/non-AIDS and unknown causes of death, stratified by demographics and HCV-related factors are shown in Table 7.2.

All-cause mortality and non-LRD rates were consistently higher in those aged 55 and over, however, LRD rates peaked in the years 35-45 at 12.0 (95% CI 9.4 – 14.7) per 1000 PYFU before tapering off in later life (45-55: 9.9 (6.9 – 12.8); >55: 6.7 (1.4 – 12.0)). LRD rates were 2-fold higher among those positive for HCV RNA compared with those negative for HCV RNA (cDR 10.1 (8.1 – 12.2) and 5.6 (2.5 – 8.6) per 1000 PYFU, respectively). LRD rates were also 2.5-fold higher in those positive for HBsAg compared with HBsAg negative (cDR 21.3 (12.9 – 29.7) and 8.3 (6.8 – 9.8) per 1000 PYFU, respectively).

Current alcohol abuse was consistently associated with higher cDRs overall and for each cause of death. LRD rates were 13-fold higher among current alcohol abusers compared with those reporting no alcohol abuse (cDR 36.8 (95% CI 18.5 – 55.0) and 2.9 (1.0 – 4.8) per 1000 PYFU, respectively). Advanced levels of liver fibrosis were also consistently associated with higher cDRs for each cause of death. Those with F4 fibrosis were at 4-fold increased risk of non-LRD/non-AIDS-related death (cDR 30.7 (21.0 – 40.5) and 7.9 (6.1 – 9.7) per 1000 PYFU, respectively) and 2.5-fold increased risk of AIDS-related death

Table 7.2 Crude Death Rates among the whole analysis population stratified by cause of death

	Cause of death				
Patient Strata	***Overall (N=670)***	***Liver-related (N=145)***	***AIDS (N=162)***	***Non-LRD/ Non-AIDS (N=233)***	***Unknown (N=130)***
Age					
< 35	31.8 (26.6 - 37.1)	4.0 (2.1 - 5.9)	11.7 (8.5 - 14.9)	11.5 (8.3 - 14.7)	4.7 (2.6 - 6.7)
35 ≤ age < 45	44.0 (39.1 - 49.0)	12.0 (9.4 - 14.7)	11.0 (8.5 - 13.5)	13.6 (10.8 - 16.4)	7.5 (5.4 - 9.5)
45 ≤ age < 55	40.6 (34.7 - 46.5)	9.9 (6.9 - 12.8)	6.7 (4.2 - 9.1)	14.2 (10.7 - 17.7)	9.9 (6.9 - 12.8)
≥ 55	75.6 (58.3 - 92.8)	6.7 (1.4 - 12.0)	12.2 (5.0 - 19.4)	36.7 (24.4 - 49.0)	20.0 (10.9 - 29.2)
Region of EuroSIDA					
South	30.9 (25.7 - 36.0)	9.7 (6.8 - 12.6)	6.7 (4.3 - 9.1)	10.6 (7.6 - 13.7)	3.9 (2.1 - 5.8)
West Central	40.9 (33.0 - 48.7)	11.6 (7.3 - 15.8)	5.0 (2.2 - 7.7)	10.3 (6.3 - 14.3)	14.0 (9.4 - 18.7)
North	81.0 (69.3 - 92.7)	17.7 (12.1 - 23.4)	11.5 (6.9 - 16.1)	35.5 (27.5 - 43.4)	16.3 (10.9 - 21.7)
East Central	22.3 (16.9 - 27.7)	4.5 (2.1 - 7.0)	3.5 (1.3 - 5.6)	10.1 (6.4 - 13.8)	4.2 (1.8 - 6.5)
East	48.1 (41.4 - 54.8)	5.9 (3.5 - 8.3)	20.5 (16.0 - 24.9)	13.8 (10.2 - 17.5)	7.9 (5.1 - 10.7)
Argentina	34.4 (17.8 - 50.9)	4.3 (0.0 - 10.2)	15.0 (4.0 - 26.1)	10.7 (1.4 - 20.1)	4.3 (0.0 - 10.2)
HCV RNA Status*					
HCV RNA Negative	32.5 (25.3 - 39.6)	5.6 (2.5 - 8.6)	6.4 (3.2 - 9.6)	13.7 (9.0 - 18.4)	6.8 (3.5 - 10.2)
HCV RNA Positive	39.1 (35.1 - 43.0)	10.1 (8.1 - 12.2)	6.7 (5.0 - 8.3)	14.3 (11.9 - 16.7)	8.0 (6.2 - 9.8)
Unknown	51.8 (45.3 - 58.3)	8.5 (5.8 - 11.2)	19.1 (15.0 - 23.1)	15.2 (11.7 - 18.8)	9.0 (6.2 - 11.7)
HBV Status*					

	Cause of death				
Patient Strata	***Overall (N=670)***	***Liver-related (N=145)***	***AIDS (N=162)***	***Non-LRD/ Non-AIDS (N=233)***	***Unknown (N=130)***
HBsAg Negative	40.3 (37.0 - 43.5)	8.3 (6.8 - 9.8)	9.6 (8.0 - 11.2)	14.8 (12.8 - 16.8)	7.6 (6.2 - 9.1)
HBsAg Positive	61.3 (47.3 - 75.3)	21.3 (12.9 - 29.7)	16.9 (9.3 - 24.4)	15.1 (8.0 - 22.2)	8.0 (2.8 - 13.2)
Unknown	38.1 (24.9 - 51.2)	4.9 (0.1 - 9.7)	8.6 (2.3 - 14.9)	8.6 (2.3 - 14.9)	16.0 (7.4 - 24.6)
Transmission Risk Group					
MSM	32.7 (22.9 - 42.6)	6.4 (2.0 - 10.8)	7.2 (2.5 - 11.9)	13.6 (7.2 - 20.0)	5.6 (1.5 - 9.7)
IDU	45.5 (41.7 - 49.3)	9.7 (7.9 - 11.5)	10.5 (8.6 - 12.4)	16.2 (13.9 - 18.5)	9.1 (7.4 - 10.9)
Other	31.0 (25.0 - 37.0)	7.4 (4.5 - 10.4)	9.6 (6.3 - 13.0)	8.7 (5.5 - 11.9)	5.3 (2.8 - 7.8)
CD4 Cell Count*					
< 200	145.5 (130.7 - 160.3)	37.1 (29.1 - 45.0)	51.7 (42.4 - 61.0)	37.5 (29.6 - 45.5)	19.2 (13.5 - 25.0)
200 ≤ CD4 < 350	41.9 (35.3 - 48.4)	8.9 (5.8 - 12.0)	6.7 (4.0 - 9.4)	15.6 (11.6 - 19.7)	10.6 (7.3 - 14.0)
350 ≤ CD4 < 500	21.1 (16.5 - 25.7)	4.0 (2.0 - 6.0)	2.6 (1.0 - 4.3)	10.3 (7.1 - 13.5)	4.2 (2.2 - 6.3)
≥ 500	16.1 (13.1 - 19.2)	2.2 (1.0 - 3.3)	1.1 (0.3 - 1.9)	7.8 (5.6 - 9.9)	5.1 (3.4 - 6.9)
HIV RNA*					
< 500	27.7 (24.6 - 30.7)	5.3 (4.0 - 6.7)	4.3 (3.1 - 5.5)	12.2 (10.2 - 14.3)	5.9 (4.4 - 7.3)
500 - 1,000	46.3 (24.8 - 67.7)	8.2 (0.0 - 17.4)	19.1 (5.1 - 33.0)	10.9 (0.3 - 21.5)	8.2 (0.0 - 17.4)
> 1,000	74.3 (65.8 - 82.8)	20.3 (15.8 - 24.9)	19.8 (15.3 - 24.3)	20.9 (16.3 - 25.5)	13.3 (9.6 - 17.0)
Alcohol Abuse*					
None	26.9 (21.2 - 32.6)	2.9 (1.0 - 4.8)	8.7 (5.5 - 12.0)	9.1 (5.7 - 12.4)	6.2 (3.4 - 8.9)
Current	110.3 (79.9 - 140.7)	36.8 (18.5 - 55.0)	34.3 (16.7 - 52.0)	27.0 (11.2 - 42.7)	12.3 (1.6 - 22.9)

	Cause of death				
Patient Strata	***Overall (N=670)***	***Liver-related (N=145)***	***AIDS (N=162)***	***Non-LRD/ Non-AIDS (N=233)***	***Unknown (N=130)***
Unknown	43.0 (39.5 - 46.6)	9.6 (7.9 - 11.3)	9.6 (7.9 - 11.3)	15.4 (13.3 - 17.6)	8.4 (6.8 - 10.0)
Liver Fibrosis*					
F0/F1	18.7 (16.0 - 21.5)	1.2 (0.5 - 1.9)	5.6 (4.1 - 7.1)	7.9 (6.1 - 9.7)	4.0 (2.7 - 5.3)
F2/F3	27.2 (15.1 - 39.2)	10.0 (2.6 - 17.4)	5.7 (0.1 - 11.3)	8.6 (1.7 - 15.4)	2.9 (0.0 - 6.8)
F4	103.8 (86.6 - 121.0)	42.4 (31.0 - 53.7)	14.1 (7.5 - 20.8)	30.7 (21.0 - 40.5)	16.6 (9.4 - 23.8)
Unknown	73.6 (66.2 - 81.1)	16.0 (12.5 - 19.6)	18.6 (14.7 - 22.4)	24.3 (19.9 - 28.6)	14.8 (11.3 - 18.2)

All rates and 95% confidence intervals per 1000 PYFU

***Time-updated variables**

(cDR 14.1 (7.5 – 20.8) and 5.6 (4.1 – 7.1) per 1000 PYFU, respectively), compared with those with F0/F1 fibrosis. The rate of LRD clearly progressed as liver fibrosis levels increased. Those with F2/F3 (cDR 10.0 (2.3 – 17.4)) and F4 fibrosis (cDR 42.4 (31.0 – 53.7)) were at 8-fold and 35-fold increased risk of LRD compared with those with F0/F1 fibrosis (cDR 1.2 (0.5 – 1.9)).

Low CD4 cell counts were also consistently associated with increased cDRs for all causes of death. The rates of all causes of mortality increased as CD4 cell count decreased with the most striking increase in mortality seen once CD4 cell counts fell below 200cell/mm^3. In those with CD4 cell counts >500cells/mm^3 the rates of AIDS and LRD were 1.1 (95% CI 0.3 – 1.9) and 2.2 (1.0 – 3.3) per 1000 PYFU, whereas for those with <200cells/mm^3 the rates were 51.7 (42.4 – 61.0) and 37.1 (29.1 – 45.0) per 1000 PYFU, respectively.

7.4.3 Temporal changes in the rate of LRD

Although LRD was one of the leading causes of death recorded over the study period, the overall incidence of LRD has declined from 23.3 (95% CI 11.2 – 35.3) per 1,000 PYFU in the year 2000 to 6.8 (0.9 – 12.8) in 2013. In univariable Poisson regression the overall incidence of LRD has declined 15% per year since 2000 (incidence rate ratio (IRR): 0.85 (0.82 – 0.89; *P*<0.0001)) (Figure 7.5). However, there was also a highly significant interaction between the incidence of LRD over time and region of EuroSIDA (*P*=0.0071), including when restricting the follow-up period to 2005 onwards when data began to be collected in Eastern Europe (*P*=0.022). This indicates that changes in the incidence of LRD over time are different depending on region of EuroSIDA.

Stratifying the analysis of LRD incidence by Western and Eastern European regions it appears that LRD rates in these groups have behaved quite differently over time. In Western Europe and Argentina the incidence of LRD has declined 16% per year since 2000 (IRR: 0.84 (95% CI 0.80 – 0.88; *P*<0.0001)). However, in Eastern Europe the incidence of LRD has remained stable over time since 2005, when data began to be collected in the region, (IRR: 0.98 (0.87 – 1.09; *P*=0.76)) (Figure 7.6).

Adjusting the model for Western Europe and Argentina for demographic factors and time-updated CD4 cell count saw the yearly decline in the incidence of LRD reduced to

Figure 7.5 Incidence of liver-related death in EuroSIDA since 2000

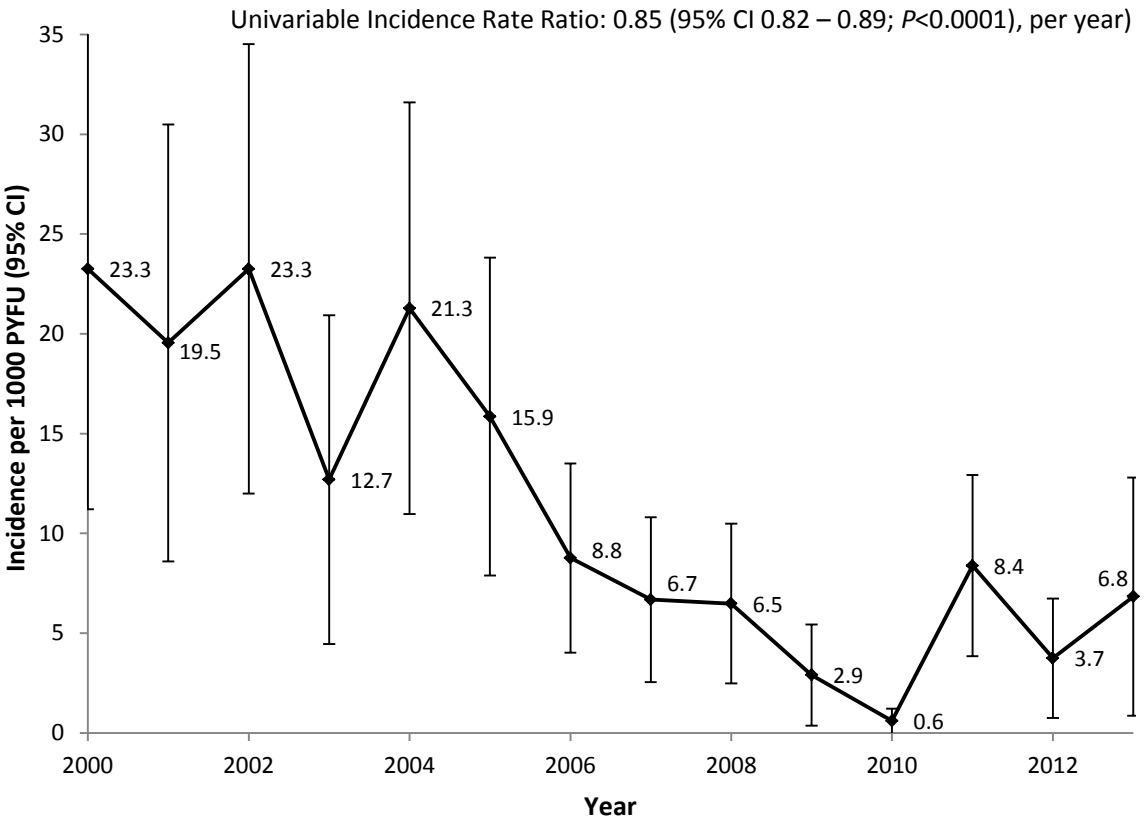


Figure 7.6 Incidence of liver-related death in EuroSIDA by region

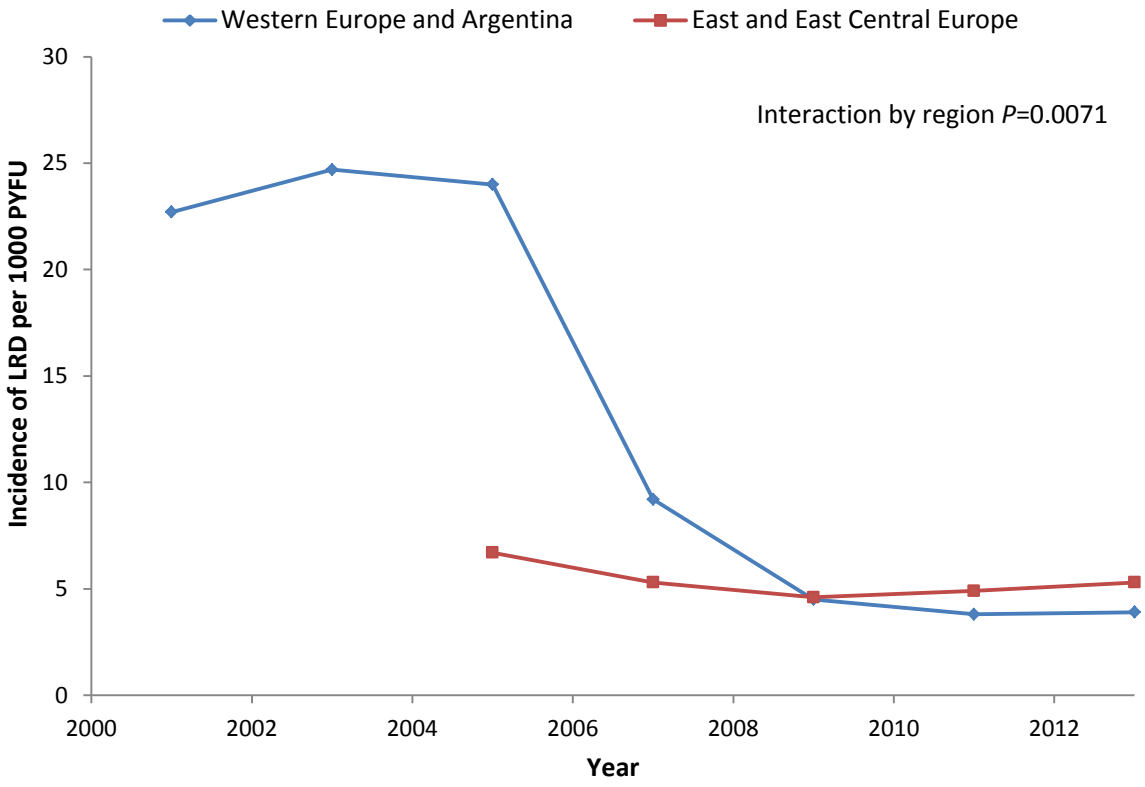


Table 7.3 Effect of CD4 cell count and liver fibrosis levels on the incidence of LRD over time in EuroSIDA

		Region	
IRR (95% CI) per year		***Western Europe & Argentina***	***Eastern Europe***
Univariable		0.84 (0.80 – 0.88; *P*<0.0001)	0.98 (0.87 – 1.09; *P*=0.76)
Adjusted:	Demographics*	0.82 (0.78 – 0.86; *P*<0.0001)	0.98 (0.88 – 1.09; *P*=0.66)
	+ CD4	0.87 (0.83 – 0.92; *P*<0.0001)	0.96 (0.86 – 1.08; *P*=0.54)
	+ Fibrosis	0.86 (0.81 – 0.91; *P*<0.0001)	1.02 (0.91 – 1.14; *P*=0.75)
	+ CD4 + Fibrosis	0.90 (0.85 – 0.96; *P*=0.0007)	0.99 (0.88 – 1.12; *P*=0.87)

***Demographics: age, sex, race, HIV transmission risk group**

13% per year (aIRR: 0.87 (0.83 – 0.92; *P*<0.0001)) (Table 7.3). Similarly, adjusting this model for demographic factors and time-updated liver fibrosis levels saw the yearly decline in the incidence of LRD reduced to 14% per year (aIRR: 0.86 (0.81 – 0.91; *P*<0.0001)). When adjusting for demographic factors plus both CD4 cell count and liver fibrosis levels the decline in the incidence of LRD reduced to 10% per year (aIRR: 0.90 (0.85 – 0.96; *P*=0.0007)).

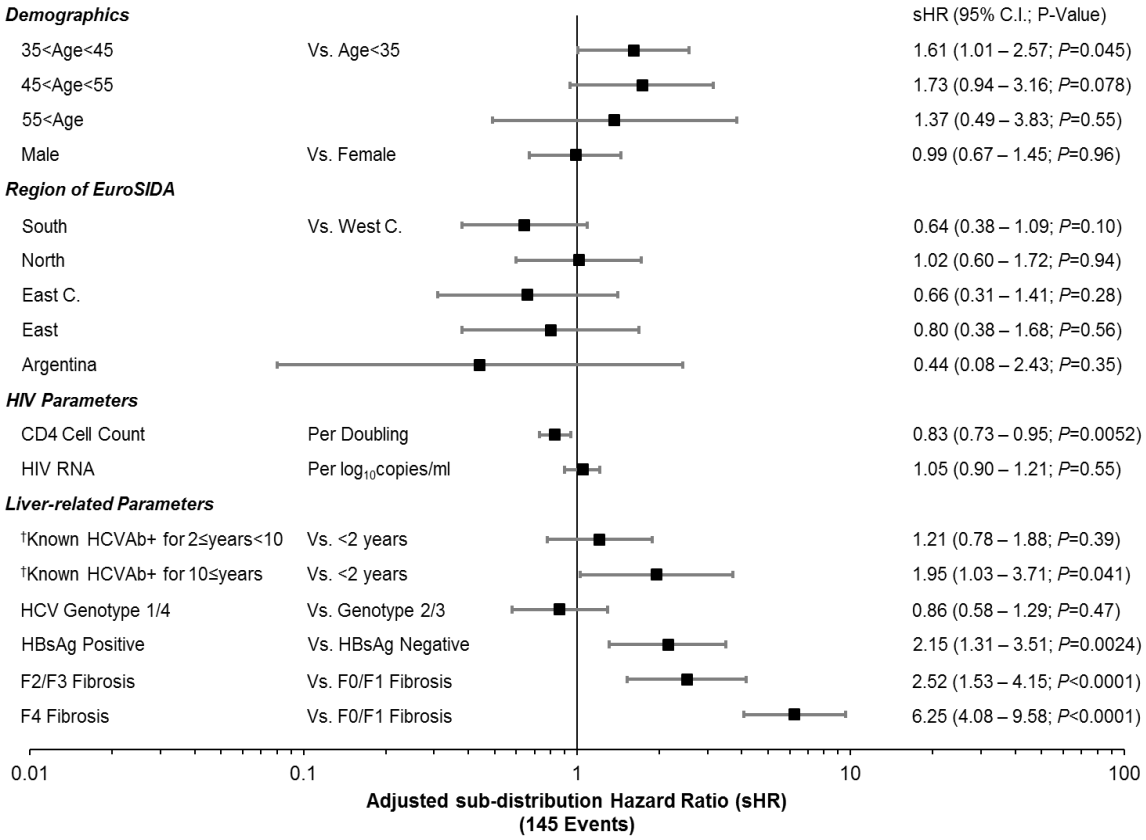
Adjusting the Eastern Europe model for demographic factors, time-updated CD4 cell count and liver fibrosis levels did not make any notable change to the stability of the incidence of LRD over time seen in this region (Table 7.3).

7.4.4 Factors associated with liver-related death

Multivariable sub-distribution hazard ratio (sHR) estimates of factors associated with the cumulative incidence of LRD from Cox proportional hazards modelling, using the Fine and Gray methodology for handling competing risks, are shown in Figure 7.7. The strongest association with LRD was seen for liver fibrosis staging. F4 liver fibrosis was associated with a 6-fold increased risk of LRD (sHR: 6.25 (95% CI 4.08 – 9.58; *P*<0.0001)), while F2/F3 fibrosis was associated with a 2.5-fold increased risk of LRD (sHR: 2.52 (1.53 – 4.15; *P*<0.0001)), compared with F0/F1 fibrosis.

HBsAg positive individuals were also at 2-fold increased risk of LRD (sHR: 2.15 (95% CI 1.31 – 3.51; *P*=0.0024)) compared with those negative for HBsAg. Those with a minimum duration of HCV infection of more than 10 years were at 2-fold increased risk of LRD (sHR: 1.95 (1.03 – 3.71; *P*=0.041)), compared with those with minimum durations of infection less than 2 years. Individuals with minimum durations of HCV infection between 2 and 10 years

Figure 7.7 Factors associated with sub-distribution hazards of liver-related death



Additionally adjusted for: gender, race, calendar year, HIV transmission risk group, previous cardiovascular events, previous diabetes diagnosis, and HCV genotype

were estimated to have 20% increased risk of LRD, however, this effect did not approach statistical significance (sHR: 1.21 (0.78 – 1.88; *P*=0.39)). Interestingly, middle age and not older age was found to be associated with an increased risk of LRD. Those aged between 35 and 45 were at 60% increased risk of LRD compared with those aged less than 35 (sHR: 1.61 (95% CI 1.01 – 2.57; *P*=0.045)). A similar effect was seen for those aged between 45 and 55 (sHR: 1.73 (0.94 – 3.16; *P*=0.078)), however, it did not quite reach statistical significance.

CD4 cell count was also strongly associated with LRD. Each doubling of CD4 cell count was associated with a 17% reduction in the risk of LRD (sHR: 0.83 (95% CI 0.73 – 0.95; *P*=0.0052)). While baseline CD4 cell count was included in the model, nadir CD4 cell count was a non-significant predictor of LRD (*P*>0.2). However, when baseline CD4 cell count was omitted from the model nadir CD4 cell count became a significant predictor of LRD, with each doubling of nadir CD4 cell count associated with a 10% reduction in the risk of LRD (sHR: 0.90 (0.83 – 0.97; *P*=0.0045)).

Interaction terms between liver fibrosis staging and either CD4 cell count or age were tested in the multivariable model and found to be non-significant (P>0.3 and P>0.7, respectively), meaning that there was no evidence to suggest the effect of liver fibrosis on progression to LRD was modified by CD4 cell count or age.

7.4.5 Factors associated with AIDS and non-AIDS/non-liver-related death

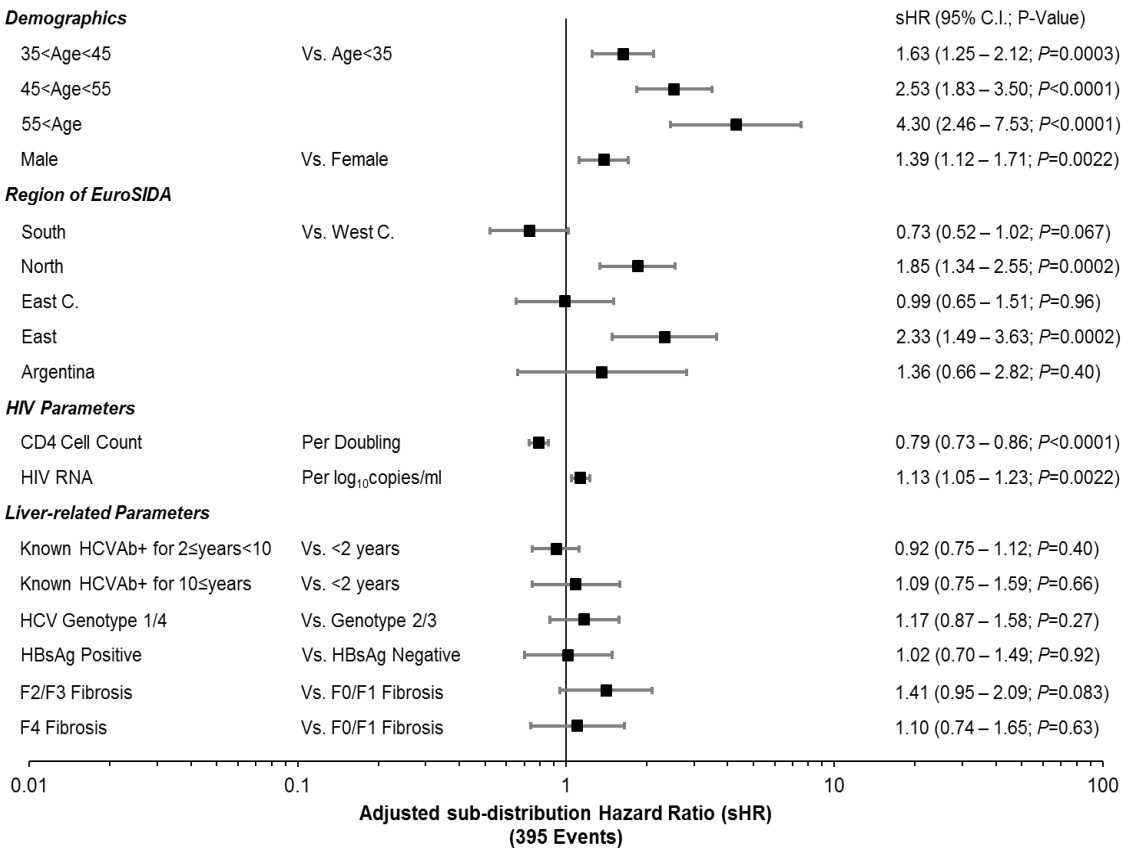
Multivariable sub-distribution hazard ratio (sHR) estimates of factors associated with the cumulative incidence of AIDS and non-AIDS/non-LRD from Cox proportional hazards modelling, using the Fine and Gray methodology for handling competing risks, are shown in Figure 7.8. Older age was strongly associated with AIDs and non-AIDS/non-LRD, with increasing risk as age increased from 35-45 (sHR: 1.63 (95% C.I. 1.25 – 2.12; P=0.0003)) to 45-55 (sHR: 2.53 (1.83 – 3.50; P<0.0001)) and greater than 55 (sHR: 4.30 (2.46 – 7.53; P<0.0001)), compared with ages less than 35. As expected, low CD4 cell count was the strongest predictor of AIDS and non-AIDS/non-LRD (sHR: 0.79 (0.73 – 0.86; P<0.0001), per doubling). Similarly, higher HIV viral load was also strongly associated with AIDS and non-AIDS/non-LRD (sHR: 1.13 (1.05 – 1.23; P=0.0022) per $\log_{10}$ higher). The male sex (sHR: 1.39 (1.12 – 1.71; P=0.0022) compared with female) and residence in Northern Europe (sHR: 1.85 (1.34 – 2.55; P=0.0002)) or Eastern Europe (sHR: 2.33 (1.49 – 3.63; P=0.0002) compared with West Central Europe), were also associated with AIDS and non-AIDS/non-LRD. However, in this multivariable model, the effects of minimum HCV infection duration and liver fibrosis staging did not approach statistical significance.

7.4.6 Cumulative incidence of liver-related death

The association between liver fibrosis staging, CD4 cell count and progression to LRD can be further illustrated by stratifying the cumulative incidence of LRD by these groups. Cumulative incidence functions for time to LRD, stratified by liver fibrosis staging, are shown in Figure 7.9. The cumulative incidence of LRD was strongly influenced by liver fibrosis stage, with highly statistically significant separation between the stratified cumulative incidence functions (P<0.0001). The 5-year probability of LRD was low in those with F0/F1 fibrosis (2.2% (95% CI 1.7 – 2.9)), but substantial in those with F2/F3 fibrosis (10.3% (7.6 – 13.5)) and higher still in those with F4 fibrosis (14.0% (10.3 – 18.3); P=0.038 for comparison of F2/F3 and F4).

Cumulative incidence functions for time to LRD, stratified by CD4 cell count and liver fibrosis staging, are shown in Figure 7.10. Due to low numbers of events and individuals in some groups fibrosis stages F2-F4 have been collected together while CD4 cell count is dichotomised at 300cells/mm^3. The cumulative incidence of LRD was strongly influenced

Figure 7.8 Factors associated with sub-distribution hazards of non-liver-related death



Additionally adjusted for: gender, race, calendar year, HIV transmission risk group, previous cardiovascular events, previous diabetes diagnosis, and HCV genotype

by liver fibrosis staging and CD4 cell count, with highly statistically significant separation between the cumulative incidence functions (*P*<0.0001). The 5-year probability of LRD was low in those with F0/F1 fibrosis and CD4 cell count ≥300cells/mm^3 (1.7% (95% CI 1.1 – 2.5)), although a little higher in those with <300cells/mm^3 (3.3% (2.2 – 4.8)). However, the 5-year probability of LRD was substantial in those with ≥F2 fibrosis and ≥300cells/mm^3 (8.6% (5.9 – 11.9)) and higher still in those with <300cells/mm^3 (15.3% (11.7 – 19.3)).

Figure 7.9 Cumulative incidence of LRD stratified by baseline liver fibrosis

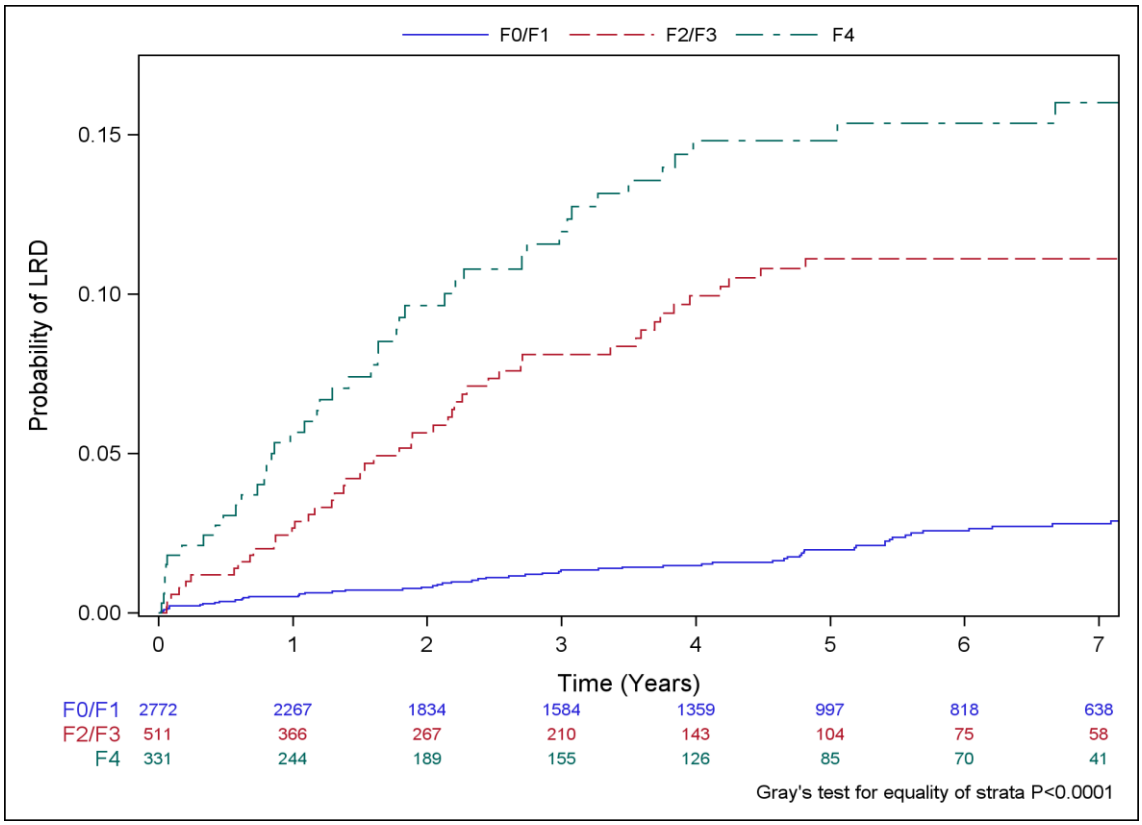
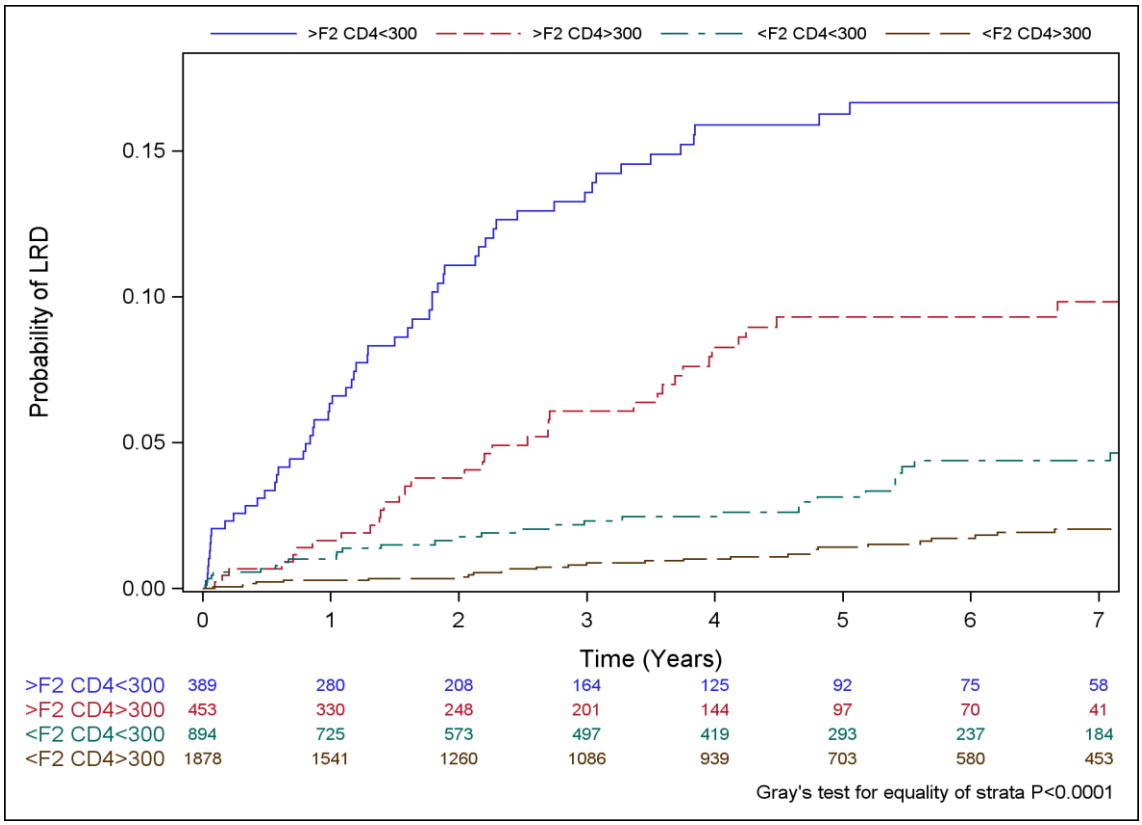


Figure 7.10 Cumulative incidence of LRD stratified by baseline liver fibrosis and CD4 cell count



7.5 Discussion

Liver fibrosis strongly associated with LRD

This chapter described causes of death among HIV/HCV coinfected individuals, temporal changes in the incidence of LRD and factors associated with progression to LRD. The main findings of this analysis show that LRD, along with AIDS, was the most common cause of death in this population, highlighting the importance of appropriate HCV disease management in order to reduce the burden of LRD among HIV/HCV coinfected individuals. LRD occurred almost exclusively among those with ≥F2 fibrosis at baseline, with cDRs for LRD 8-fold and 35-fold higher among those with F2/F3 and F4 fibrosis compared with F0/F1 fibrosis. Further, the 5-year probability of LRD was low among those with F0/F1 fibrosis, but substantial at 10.3% in those with F2/F3 fibrosis and as high as 14.0% in those with F4 fibrosis.

Interestingly, high levels of liver fibrosis were not only associated with higher rates of LRD. AIDS and non-AIDS/non-LRD crude death rates were also substantially higher among those with F4 fibrosis, suggesting that high levels of liver fibrosis may be associated with a number of poor clinical outcomes not just those considered to be linked to the liver. However, in multivariable analysis while traditional risk factors for progression to AIDS and non-AIDS/non-LRD were present, such as low CD4 cell count, high HIV viral load and older age, liver fibrosis staging was not found to be a significant predictor. Clearly there are many competing risks encountered by HIV/HCV coinfected individuals and identifying those most likely to progress to LRD is essential when deciding who to treat with new DAAs for treatment of HCV.

These findings are in agreement with a recent study from the Veterans Aging Cohort Study. Including 4,280 ART-treated HIV/HCV coinfected individuals over a follow-up period of 1997-2010 the authors found that low levels of liver fibrosis (Metavir F0/F1), as measured by the FIB-4 index, were associated with a minimal 5-year risk of end stage liver disease (ESLD). However, the 5-year risk of ESLD increased to 17% for those with F3/F4 fibrosis[665]. The agreement of these two large studies suggests that liver fibrosis staging should be considered the most important risk factor for LRD. Further, after assessing the risk of death from non-liver-related causes, treatment with new DAAs should be prioritised for those with ≥F2 fibrosis.

Current European HCV treatment guidelines recommend deferring HCV treatment in those with F0/F1 liver fibrosis, while treatment is encouraged for those with significant liver fibrosis (≥F2)[416]. Given the very low risk of LRD seen for those with F0/F1 fibrosis in this

study, this is a stance that is strongly supported by the findings presented here. However, these recommendations also require that treatment of HCV remains successful in those with higher levels of liver fibrosis and that fibrosis levels may regress when HCV RNA is eradicated, leading to fewer LRDs. Although DAA therapy is in its infancy and will require long-term follow-up to detail the full benefits of treatment, data is available from more traditional interferon-based treatment.

In 2009 *George et al* reported on 150 HCV-monoinfected individuals followed-up for 5 years after achieving SVR from HCV treatment. Those with significant liver fibrosis at SVR had subsequent liver biopsies performed after 4 years of follow-up and 82% had improved liver fibrosis scores, while 92% improved at least one component of liver inflammation[666]. However, the authors also state that there were 16 individuals with cirrhosis prior to treatment and that two of these (12.5%) developed HCC even after achieving SVR. Therefore, although there is evidence that liver histology improves after SVR, there remains a residual risk of HCC, especially in those with cirrhosis[666].

In 2007 *Bruno et al* reported retrospective data on 920 HCV-monoinfected individuals with cirrhosis between 1992 and 1997, showing that those who did not achieve SVR were at 7-fold increased risk of LRD and 2.5-fold increased risk of HCC[667]. While these data are based on HCV-monoinfection, the lower rates of SVR from interferon-based treatment typically seen for HIV/HCV coinfected individuals appears to be a thing of the past with new DAA therapies[668;669].

CD4 cell count and HBV coinfection strongly associated with LRD

In this study, in addition to significant liver fibrosis, concurrent HBV coinfection, lower CD4 cell count and minimum duration of HCV infection were all associated with LRD in multivariable analysis. Further, when baseline CD4 cell count was omitted from the model, nadir CD4 cell count was also strongly associated with LRD. Taken together these findings suggest that efforts to reduce late presentation of coinfected individuals are essential so that current treatment guidelines can be employed in HIV/HCV coinfected individuals. ART should be initiated early during the course of HIV progression and in the case of concurrent HBV coinfection should contain potent HBV-active treatment[416]. These recommendations, if applied correctly, should reduce the risk of prolonged time spent with low CD4 cell counts and uncontrolled HBV coinfection, which would reduce the risk of progression to LRD.

Although the interaction between CD4 cell count and liver fibrosis staging did not approach statistical significance during multivariable modelling, there was some evidence from the stratified cumulative incidence of LRD to suggest that the risk of LRD was higher among

those with ≥F2 fibrosis when baseline CD4 cell counts were below 300cells/mm^3. Previous studies have consistently shown that low CD4 cell count, high HIV viral load and circulating HBV DNA are all associated with rapid progression of liver fibrosis levels[414;670;671]. Each of these risk factors can be controlled by initiating appropriate cART early in coinfected individuals, potentially reducing progression of liver fibrosis levels and therefore the need for expensive DAA therapy, which highlights the public health problems brought about by late presentation of HIV/HCV coinfection.

Interestingly, ages between 35 and 45 were found to be associated with increased risk of LRD in this study, while ages between 45 and 55 were associated with LRD with borderline statistical significance. However, the effect sizes were comparable for those aged 35-45, 45-55 and >55, which may suggest that the risk of LRD is increased but stable after the age of 35. Similarly, minimum duration of HCV infection was associated with LRD, with those diagnosed for ≥10 years at increased risk. As expected, older age was strongly associated with death from non-AIDS/non-LRD and unknown causes. In terms of competing risks this may serve to lower the risk of LRD in later life as death from other causes becomes more prominent, which may explain the flat association between age after 35 and LRD.

Alcohol abuse and LRD rates

Coinfected individuals reporting current alcohol abuse were found to have 13-fold higher cDRs for LRD. However, data on alcohol abuse were only added to EuroSIDA in 2010 and 40% of the study population had no alcohol abuse data available. Collection of data on alcohol abuse is often difficult in the clinical setting. Many factors are believed to affect the quality and reliability of data on alcohol abuse including, the characteristics of the participants, the question being asked and the manner of the assessor[672]. This and the fact that many individuals had missing data for alcohol abuse, means that the association between alcohol abuse and LRD should be interpreted with caution.

Estimation of Fine and Gray competing risks models does not allow for simple interpretation of time-updating variables, while the aims of this study were to identify medium to long term risk factors associated with LRD, rather than the short term associations represented by time-updating covariates. Therefore, the effect of alcohol abuse on LRD was not assessed in the competing risks model. However, alcohol consumption is a known contributor to the rapid progression of liver fibrosis and acts synergistically with HCV infection to speed up the process further[393;673].

HIV/HCV coinfected individuals should be warned of the potential for accelerated liver damage caused by drinking excessively during routine clinical care, with the aim to reduce alcohol intake and prevent the rapid development of fibrosis[674]. However, the reality is that many coinfected individuals belong to difficult to treat populations engaging in active IDU and alcohol abuse[675;676], where optimal clinical management is not always possible.

The incidence of LRD over time

LRD rates were found to have decreased significantly over the period of follow-up of this study. This finding is in agreement with other recent studies mentioned in the introduction, which report reducing mortality rates among HIV-positive individuals from 2000 to 2010, particularly for LRD[372;373]. However, this study adds to this topic by presenting data on a large number of HIV/HCV coinfected individuals. While the overall trend was for reducing LRD rates, when stratifying by region of EuroSIDA it became clear that the reduction is driven by a fall in the rate of LRD in Western Europe and Argentina seen since 2005. Data available in Eastern Europe show that the rate of LRD has remained stable since the region was added to EuroSIDA in 2005.

The reduction in the incidence of LRD seen in Western Europe was partly explained by changes in CD4 cell count and liver fibrosis levels over time. When adjusting for demographic factors, time-updating CD4 cell count and liver fibrosis stage the decline in LRD incidence reduced from 16% to 10% per year, a reduction of 38%. EuroSIDA is an open cohort that continues to recruit participants. These results suggest that improvements in CD4 cell counts over time, as a result of improvements in the efficacy and tolerability of cART regimens, along with lower fibrosis stages in newly recruited individuals, has contributed to the reduction in the incidence of LRD in Western Europe. However, a large proportion of this reduction over time cannot be accounted for in this analysis. One possible explanation could be a cohort effect where the individuals most likely to die of LRD do so early during follow-up leaving a relatively low-risk population with regards to LRD.

Eastern Europe is well-known to have a large number of HIV/HCV coinfected individuals and accounted for 31.0% of the total study population[404]. However, the region accounted for just 15.9% of the LRDs recorded in this study. In comparison 49.4% of all AIDS-related deaths occurred in Eastern Europe, which illustrates the competing risks these individuals face. However, it is also highlights the imbalance in access to HIV treatment and care by region of Europe that has been reported previously in EuroSIDA[677]. Adjusting for CD4 cell count and liver fibrosis levels over time showed that 38% of the 16% reduction in the rate of LRD per year in Western Europe and Argentina was attributed to CD4 cell count and liver

fibrosis levels. However, adjusting for the same variables in Eastern Europe made little difference.

With this in mind it is hard to imagine a scenario where wide-ranging access to expensive DAA therapies will be possible in Eastern European regions. Although the region contains the highest prevalence of HIV/HCV coinfection in Europe[678], a comprehensive cART program aiming to reduce the excess AIDS-related death in the region is likely to see more benefit to public health than introduction of DAAs. Further, increasing CD4 cell counts with appropriate cART may also lead indirectly to lower rates of LRD[387].

HCV reinfection, sustained virologic response and all-cause mortality

Unlike other viral infections, clearance of HCV infection with or without treatment infers only partial protection against reinfection[468]. Many studies have documented a high incidence of reinfection with HCV after viral clearance, particularly among IDUs[472]. In a recent EuroSIDA study 18% of all coinfected individuals that achieved spontaneous clearance became reinfected within a few years, with 94% of those having acquired HIV via IDU[679]. However, reinfection is not restricted to IDUs. Studies have also documented reinfection rates as high as 15% among MSM[474]. Consequently, in the setting of treatment prioritisation for DAA therapy, risk behaviour associated with reinfection must be a key consideration before initiating expensive treatment regimens.

While HCV has traditionally been considered a disease of the liver, data is beginning to emerge which suggests that HCV eradication may lead to a reduction in all-cause mortality[680]. A recent study of a large number of HCV seropositive and seronegative individuals found that those positive for HCV were at increased risk of death from hepatic and extrahepatic diseases[681]. Potential reasons for the association between HCV infection and all-cause mortality include complications associated with fibrosis and cirrhosis or an inflammatory effect of circulating HCV RNA[682]. This study was designed to describe factors associated with progression to LRD among HIV/HCV coinfected individuals with chronic HCV infection prior to treatment for HCV. Therefore, it was not possible to determine the effect of HCV RNA eradication on overall mortality. However, in exploratory analysis I also found consistently higher cDRs among those HCV RNA positive compared with those HCV RNA negative.

This emerging evidence, taken together with observed higher SVR rates among those with low levels of liver fibrosis and the potential reduction of onward transmission[446;659], may mean that eventually it will be beneficial to treat all HIV/HCV coinfected individuals regardless of fibrosis staging[683]. In addition, recent analysis of the cost-effectiveness of

treating all HCV monoinfected individuals shows that it may indeed be a cost saving intervention[461;684]. However, while the pathways by which active HCV infection may be linked with non-LRD remain unclear and treatment remains prohibitively expensive, prioritisation of those at greatest risk of LRD is still necessary. The pathogenesis of liver disease varies among individuals and those who are not initially prioritised for new DAA therapies should maintain clinical care so that they may be monitored frequently for signs of progression of liver fibrosis.

7.5.1 Limitations

The main limitation of this study was that, due to limited power, it was not possible to compare the individual stages of liver fibrosis which were grouped as F0/F1, F2/F3 and F4 fibrosis. Further, as the fibrosis data in this study are collected from a combination of clinical procedures and biomarkers, consensus on the definitions of the stages of fibrosis is difficult to attain. However, cut-off points at either end of the scale, low levels of fibrosis or cirrhosis, are relatively well-defined[589]. In particular, the APRI score, which is where the majority of fibrosis data are taken from in this study, has been validated in a number of studies[425;685]. However, misclassification of liver fibrosis levels in this study would make differentiation between the fibrosis stages less precise, which would lead to an under estimation of the differences between fibrosis stages with respect to progression to LRD.

This study clearly identified a significant relationship between lower CD4 cell counts and progression to LRD. However, due to few LRDs among those with high CD4 cell counts it was not possible to perform analysis to determine the optimum time to start cART in to order to prevent progression to LRD.

A further limitation of this study is that data were not complete at baseline. Multiple imputations were performed in order to impute the missing data; however, this is a well-known statistical technique that produces unbiased estimates when data are missing completely at random (MCAR) or missing at random (MAR)[522]. The missing data in this analysis were HCV RNA, HCV genotype, HBsAg and fibrosis staging. Section 4.4.1 of this thesis showed that missing HCV RNA data was associated with Eastern Europe. Eastern Europe is more likely to have missing data than Western Europe due to differences in the quality of clinical management. However, this does not mean that the underlying distribution of people positive or negative for HCV RNA would be different in this region. Therefore, it is reasonable to assume these data are MAR. Similarly, any differences in the underlying distributions of HBsAg, HCV genotype and fibrosis staging between Eastern and Western Europe are likely to be explained by the observed variable region of EuroSIDA. Consequently, I believe it is reasonable to assume these data are MAR. In addition, the

amount of missing data was low for key variables in this analysis, such as liver fibrosis staging (21.9% missing).

Alcohol abuse data was only available from 2010 onwards, meaning that for a large number of individuals this data was missing and it could not be included in competing risks modelling. Further, the quantitative nature of this data means that the amount of alcohol being consumed was not recorded, just whether the individual was considered an alcohol abuser. Therefore, it is not possible to determine a level of alcohol consumption that increases the risk of liver fibrosis and progression to LRD. Finally, minimum duration of HCV infection was found to be associated with progression to LRD in this study, calculated using each individual's first recorded positive HCVAb or HCV RNA test result. However, this method is likely to underestimate the true duration of HCV infection in this population as seroconversion likely occurred around the same time as HIV infection.

7.5.2 Conclusion

New DAAs for treatment of HCV infection appear ready to offer impressive outcomes with better toxicity profiles than historical treatments. However, the costs of treatment will necessitate prioritisation of those at the greatest risk of LRD for therapy. In this regard, after considering the risk of death from competing risks and of potential reinfection, those with significant liver fibrosis (≥F2) should be prioritised for treatment with DAA therapy. Further, as part of a comprehensive strategy to prevent LRD in HIV/HCV coinfected individuals, greater emphasis should be given to identifying coinfected individuals as soon as possible. Coinfected individuals may then start cART early in the course of HIV infection, which in the case of concurrent HBV infection includes potent HBV active treatment, to prevent rapid progression of liver fibrosis and reduce the need for expensive DAA therapy.

Chapter 8

A validated prognostic score for estimating the risk of liver-related death among HIV/HCV coinfected individuals

8.1 Introduction

The analysis in the previous chapter identified a number of factors that were associated with progression to liver-related death (LRD) among HIV/HCV coinfected individuals. Although useful, interpretation of these individual risk factors may prove to be difficult in the clinical setting due to interplay between the individual risk factors. Therefore, it may be beneficial to condense the information into a single prognostic score which can predict an individual's risk of progression to LRD.

Prognostic scores have been developed in many areas of clinical research. The main aim of developing tools such as these is to establish a single, directly comparable among individuals, statistic from a group of independent variables which have an effect on the course of disease or outcome of interest. One well-known prognostic score is the Framingham risk score, developed to predict progression to coronary heart disease (CHD) in the general population[686].

The Framingham risk score takes information derived from a population study associating progression to CHD with blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, diabetes, smoking status and age, and combines them into one single score from which each individual, or their clinician, can estimate their 10-year risk of CHD and compare themselves to an average person of a similar age[686]. Importantly, the authors of the score provide easy to follow step-by-step instructions on how to calculate the risk score based on clinical markers, without the need for detailed knowledge of the background statistical process. It is this step that I want to emulate here so that the information derived in the previous chapter can be readily used in the clinical setting.

The c-statistic

The receiver operating characteristic (ROC) curve provides a global standardised method for comparing the accuracy of markers and prognostic scores in predicting binary events.

The ROC curve plots the sensitivity of a prognostic score against one minus its specificity (1-specificity). In other words, the curve plots the true-positive rate against the false-positive rate of a marker of a binary outcome[687]. Plotting the ROC curve then allows for calculation of the area under the ROC curve, which is known as the c-statistic[687].

The c-statistic allows direct comparison between independent prognostic scores in order to compare their ability to predict an event. A c-statistic of 1 indicates perfect identification of those who will and won't progress to an event. A c-statistic of 0.5 indicates that the marker or score performs no better than chance at determining progression to the event[687]. Consequently, the c-statistic takes values between 0.5 and 1, with improving performance as the value moves towards 1. As a rule of thumb a marker with a c-statistic greater than 0.7 is considered to have reasonable predictive ability, above 0.8 strong predictive ability, and above 0.9 excellent predictive ability[687].

8.1.1 Prognostic scores in HIV- and HCV-related research

8.1.1.1 The VACS Index

A number of prognostic risk scores have been developed in HIV- and HCV-related research. In 2013 the veterans aging cohort study (VACS) group set out to explain excess mortality among HIV-positive individuals taking cART. For HIV-positive individuals taking cART, AIDS-defining events are rare and HIV viral loads are usually very low or undetectable. However, these individuals continue to have excess mortality in comparison to demographically matched controls without HIV. Further, the excess mortality does not appear to be explained simply by differences in CD4 cell count[688-690].

In an attempt to predict mortality among HIV-positive individuals taking effective cART the VACS study group developed two prognostic scores for predicting all-cause mortality, the VACS Index and the Restricted Index. The Restricted Index contained the traditional HIV-associated risk factors CD4 cell count, HIV viral load and age, while the VACS index also included haemoglobin, a FIB-4 estimation of liver fibrosis staging, eGFR and whether an individual had HCV infection[688]. For each index an individual's score is calculated by summing the appropriate score contributions associated with their demographic and clinical characteristics.

The results of the study show that higher scores from the VACS Index and the Restricted Index were associated with progression to all-cause mortality[688]. Further, the VACS Index outperformed the Restricted Index at discriminating between those who died and did not die after 5-years of follow-up. The VACS Index had a c-statistic of 0.78 in the derivation

cohort and 0.82 in the validation cohort, while the Restricted Index had c-statistics of 0.72 and 0.78, respectively[688]. As the VACS Index outperformed the Restricted Index the authors concluded that accounting for multiple organ system injury, including the liver and kidneys by using fibrosis staging and eGFR, allowed for more accurate identification of HIV-positive individuals taking cART at increased risk of death compared to the general population[688].

One of the strengths of the VACS index is that it was validated in an external cohort. The validation took place in the antiretroviral treatment cohort collaboration (ART-CC), which is a set of six HIV cohorts independent of the VACS[688]. Validation of prognostic scores is important to show that they are generalizable to the general population. It is possible that a prognostic score developed in one cohort will not accurately reflect the risk of an event in another cohort because there are underlying differences in the population characteristics. For this reason it is usually expected that the ability of a prognostic score to predict an event will be somewhat lower in a validation cohort compared to the derivation cohort. However, the VACS index actually performed slightly better in the validation cohort, illustrating its excellent generalisability[688].

8.1.1.2 Child-Pugh and MELD scores

A number of prognostic scores exist to quantify the risk of mortality in the general population in individuals with cirrhosis. The Child-Pugh score, originally derived as the Child score in the 1960s, has five components which were selected empirically based on clinical expertise[691-693]. The five components include 3 continuous variables (bilirubin, albumin and prothrombin) and 2 quantitative variables (the degree of encephalopathy and ascites) which are believed to reflect the function of the liver (Table 8.1)[691-693]. The Child-Pugh score is calculated by summing the points associated with each component for a patient's profile.

The main application of the Child-Pugh score has been as a descriptive tool at the bedside to quantify an individual's risk status[693]; however, it has also been used as patient selection criteria for hepatocellular carcinoma surgical removal or for extrahepatic surgery[694;695]. In addition, the Child-Pugh score has been shown to have prognostic value in the setting of liver surgery, alcoholic cirrhosis, HCV-related cirrhosis and subclinical encephalopathy[696-699].

Table 8.1 The Child-Pugh score[693]

Points	***1***	***2***	***3***
Encephalopathy	None	Minimal	Advanced
Ascites	Absent	Controlled	Refractory
Bilirubin (µmol/l)	<34	34 - 51	>51
Albumin (g/l)	>35	28 - 35	<28
Prothrombin (s)	<4	4 - 6	>6

Although the Child-Pugh score is simple to calculate and has been shown to have many useful applications, it is often considered limited by the fact the components were chosen empirically and that they are all weighted equally[693]. The MELD score, which was developed to predict survival in cirrhotic patients following transjugular intrahepatic portosystemic shunt (TIPS), attempts to address these issues[700]. The MELD score was derived using a multivariable Cox proportional hazards model to identify variables which had an independent effect on survival. The current score is calculated based on three continuous variables (creatinine, bilirubin and international normalised ratio (INR)) plus a constant (Equation 8.1)[693].

Equation 8.1 The MELD score formula[693]

$$MELD = 9.6log_e(creatinine\ mg/dl) + 3.8log_e(billirubin\ mg/dl) + 11.2log_e(INR) + 6.4$$

The MELD score has since been used for prioritising cirrhotic patients for the allocation of liver grafts and determining the optimal indication for liver transplantation and has been validated in many independent samples[693;700;701]. However, the MELD score does not allow for direct estimation of an individual's probability of survival. Survival probabilities must be computed from the survival function or looked up using a reference normogram. Unfortunately, this means the MELD score is often not considered applicable for use at the bedside in a clinical setting[693;700].

The Child-Pugh and MELD scores have been compared in many settings with regards to short term mortality. In general they appear to display similar levels of discrimination, with c-statistics ranging from 0.65 to 0.85, indicating reasonable to strong predictive capabilities (Table 8.2)[702-705]. However, none of these studies included HIV or HCV positive individuals and to my knowledge these scores have never been validated in these populations. They instead focus on individuals from the general population with advanced liver disease or cirrhosis.

Table 8.2 Prognostic ability of the Child-Pugh and MELD scores as measured by the c-statistic

Study population (Year)	*N. included (deaths)*	*Study endpoints*	*c-statistic*	
			Child-Pugh	*MELD*
TIPS (2002)[702]	475 (230)	1-month mortality	0.78	0.73
		3-month mortality	0.70	0.72
		1-year mortality	0.66	0.66
TIPS (2003)[703]	162 (81)	1-month mortality	0.71	0.72
		3-month mortality	0.67	0.73
		1-year mortality	0.74	0.73
Cirrhosis (2003)[704]	3,437 (412)	3-month mortality	0.76	0.83
Liver disease (2004)[705]	1,611 (321)	3-year mortality	0.83	0.79

8.1.2 Prioritisation of direct-acting antiviral therapy for HCV

The new direct-acting antivirals (DAA) for HCV were discussed in detail in Section 2.2.6 of the introduction to this thesis. However, the anticipated costs of new DAAs for treatment of HCV are expected to approach €90,000 per individual treatment, with the expectation that all-oral regimens will be more expensive still[2]. Therefore, a number of studies have turned their attention to the cost-effectiveness of these new therapies. Two recent studies in 2014 by *Younossi et al* and *Leleu et al* have used Markov chain models to simulate the progression of liver-disease among HCV-monoinfected individuals[684;706].

Using sustained virologic response (SVR) rates estimated from clinical trials data, the *Younossi* study found that treating HCV-monoinfected individuals with all-oral interferon-free DAA regimens drastically reduced the number of individuals developing advanced liver disease[706]. The reduced lifetime costs associated with fewer people progressing to advanced liver disease meant that the authors concluded that treating all HCV-monoinfected individuals would be a cost effective strategy in the long term. The authors estimated that treating all individuals with all-oral DAAs would lead to an effective cost of each quality adjusted life year added (QALY) of $18,391, compared to interferon-based triple therapy. This means that each additional year of life that would have otherwise been lost in the absence of treatment will cost $18,391[706]. In the United States treatments are considered to be cost effective up to $50,000 per QALY[706]. However, the study also reports that a fibrosis staging guided approach would be the most cost effective strategy. When all-oral DAA regimens were given only to those with F2-F4 liver fibrosis the effective cost of each QALY reduced to $17,529[706].

The *Leleu* study followed a very similar methodology but only considered treatment regimens including the recently approved DAA sofosbuvir. Here the authors also noted that vast reductions in expensive-to-treat advanced liver diseases lead to generally acceptable levels of cost effectiveness for sofosbuvir treatments, in comparison to interferon-based triple therapy[684]. The authors concluded that sofosbuvir-based treatment would lead to an effective $40,653 cost per QALY added among individuals with F0 fibrosis. However, this figure was $31,348 among those with F1 fibrosis and reduced to $17,651, $11,359 and $12,080 among those with F2, F3 and F4 fibrosis, respectively[684]. Treating individuals with more advanced levels of fibrosis becomes more cost effective as they are more likely to progress to advanced liver disease without therapy, which would necessitate expensive care and may lead to LRD.

Current European HCV treatment guidelines tend to reflect these findings as they recommend treatment for people with ≥F2 liver fibrosis while suggesting treatment may be postponed in those with <F2 fibrosis[416]. However, they do not address how patients should be prioritised when treatments are restricted due to costs or supply issues. The cost of treatment with new DAAs means that cost restrictions are likely to occur in many settings, not just those traditionally seen as resource-limited. Earlier this year the National Institute for health and Care Excellence (NICE) approved the use of sofosbuvir and simeprevir for treatment of HCV in the UK and concluded that they were cost-effective interventions[707]. However, for the first time since NICE started making cost-effectiveness recommendations the NHS postponed approval of these drugs on the basis that the upfront costs were currently too high to bear, regardless of the fact they were considered cost-effective in the long-term[708].

Given that the costs of DAAs are proving to be prohibitive in the UK, it is certain that many other countries and regions will struggle to meet the upfront costs of treatment in the short-term. Two recent studies by *Obach et al* have attempted to provide guidance to those working in the resource-limited setting with regards to who should be prioritised for treatment for HCV in order to have the greatest impact on public health[709;710]. These studies also used Markov chain models to estimate the effect of different treatment strategies on the outcome of those infected with HCV. The authors state that treating HCV-monoinfected individuals with ≥F2 fibrosis is cost-effective in Egypt, but that assuming new DAAs will be more readily available in a few years, those with F1 fibrosis should have treatment delayed[709]. In addition, using data from Egypt, Thailand and Ivory Coast the authors conclude that when the number of treatments available per year is fixed, whether interferon-free or interferon-based, the number of life years saved with treatment in the

countries listed above increases by 3.9%, 15.3% and 11.0% when restricting treatment to those with F3 or F4 fibrosis compared to treating those with ≥F2 fibrosis, respectively[710].

When considering HIV/HCV coinfection the issue of HCV treatment prioritisation becomes somewhat more complicated. Commentators and guidelines now suggest that, due to excellent and comparable efficacy of DAA treatments for HCV monoinfected and HIV/HCV coinfected individuals, HIV infection should no longer be considered a barrier to HCV treatment[416;711]. However, clinicians must bear in mind HIV- and HCV-related factors when considering the risk of progression to LRD. Liver fibrosis is one of the key markers of progression to LRD, but low CD4 cell counts as a consequence of HIV are known to contribute to rapid progression of liver fibrosis as well as being strongly linked with AIDS-related death[414;671]. Consequently, analysis of competing risks is essential when deciding who to prioritise for treatment with new DAA therapies.

8.2 Aims

The aims of this analysis were to identify factors associated with progression to LRD among HIV/HCV coinfected individuals and to create a simple, easily applicable prognostic score to identify those at the greatest risk of LRD. A prognostic score that can be calculated with ease by a clinician or HIV/HCV coinfected individual. Creating such a score will add to the body of research on HIV/HCV coinfection by making it possible to directly compare individual risk profiles with respect to progression specifically to LRD in the presence of competing HIV-related risks. In instances where new DAA treatments for HCV are limited by costs or availability to treatments, the prognostic score will aid clinicians when making difficult decisions on which HIV/HCV coinfected individuals should be prioritised for therapy.

8.3 Methods

8.3.1 Patient selection

The D40 update of the EuroSIDA database included 18,914 HIV-positive individuals from 107 centres across Europe, Israel and Argentina. Figure 8.1 shows the breakdown of how individuals were selected for inclusion in this study. All EuroSIDA patients under prospective follow-up with documented HIV/HCV coinfection, as evidenced by a positive HCVAb test, and follow-up available after the 1st January 2000 were eligible for inclusion in this study. The D40 EuroSIDA database included 16,423 individuals with known HCVAb status, of whom 4,878 were positive and 4,011 had follow-up data recorded after 1st January 2000.

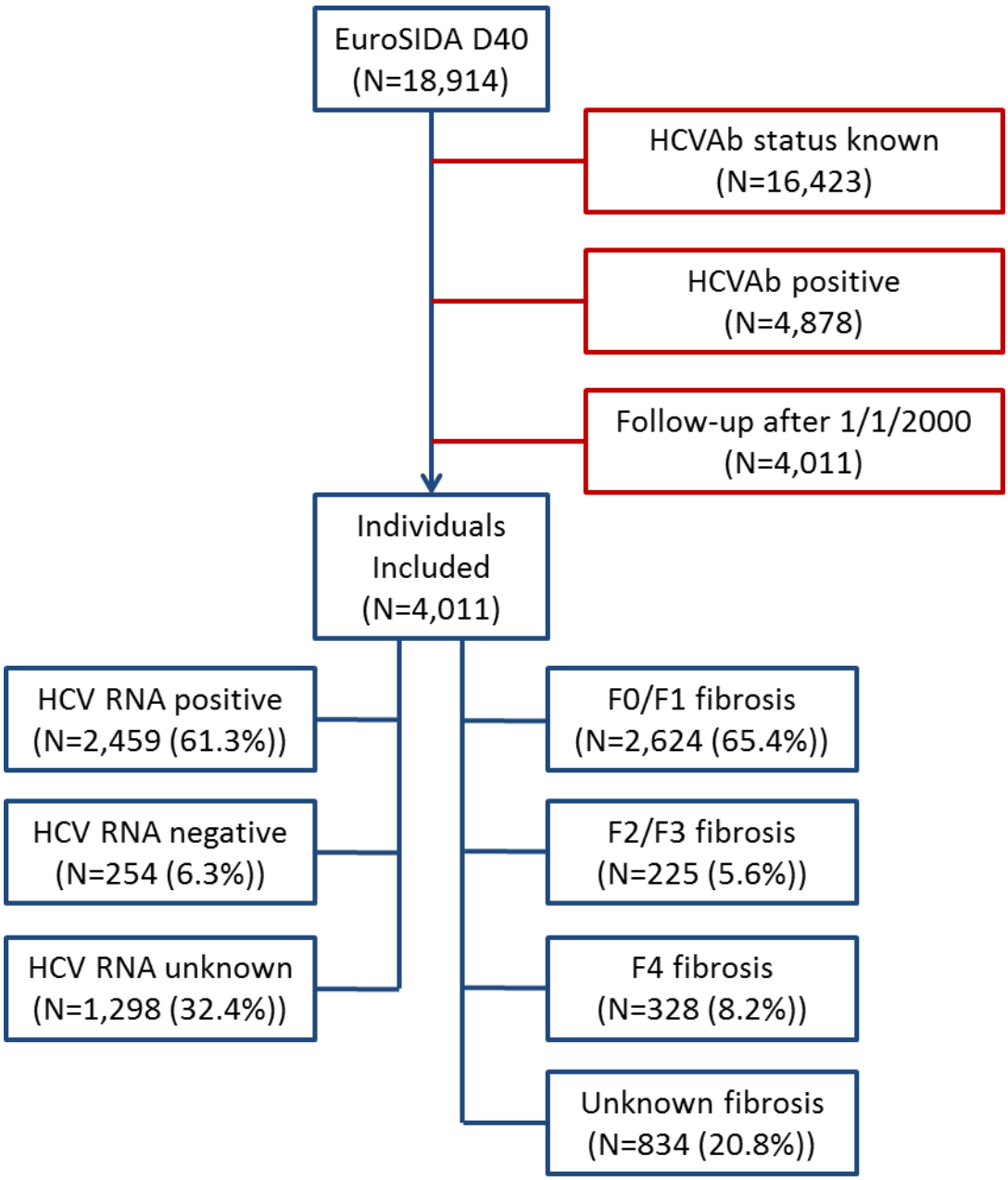
Follow-up prior to 1st January 2000 was excluded from this study as I wanted the study population to reflect the current state of clinical management of coinfected individuals. Follow-up prior to 1st January 2000 would likely include individuals receiving what today would be considered sub-optimal antiretroviral therapy. As the main aim of this study was to create a prognostic score to help prioritise who should receive treatment with new DAA drugs for HCV, follow-up was also censored at the date of starting interferon-based treatment for HCV.

8.3.2 Statistical methods

Causes of death in this analysis were classified using the CoDe methodology described in the methodology chapter of this thesis[511;512]. For the purpose of analysis deaths were classified into the following categories: LRD and non-LRD. Non-LRD includes causes of death such as AIDS, bacterial infections, cardiovascular events, drug and accidental death, and cancer. However, hepatocellular carcinoma is included in the LRD category along with liver failure, cirrhosis or complications as a result of HCV or HBV infection.

A combined estimated definition of liver fibrosis staging was used throughout this analysis according to the Metavir scoring system[420] (see Chapter 2 Section 2.2.5.3 for a description of fibrosis staging). F0/F1 fibrosis was defined from validated liver biopsy, Fibroscan® measurements <7.6Kpa, an APRI score <1.5 and hyaluronic acid (HA) measurements <100ng/ml. F4 fibrosis was defined from validated liver biopsy, Fibroscan® measurements >12.5Kpa, and APRI score >2 and HA measurements >250ng/ml, in line with previous EuroSIDA work and other studies of HCV infection[660-662].

Figure 8.1 Analysis population inclusion criteria and baseline HCV RNA and liver fibrosis status



Where more than one fibrosis measurement was available for each individual at each time point they are prioritised in the order given above. For example, if an individual had both liver biopsy and Fibroscan® data available at one time point; the liver biopsy would take precedence.

Throughout this analysis baseline was defined as 1st January 2000, entry into EuroSIDA, or the first available fibrosis measurement, whichever occurred later. Cox proportional hazards regression, adopting the Fine and Gray methodology of competing risks, was used

to describe factors associated with progression to LRD[519]. The competing risks accounted for were death from causes other than LRD and loss to follow-up, defined as no contact with a EuroSIDA clinical site in the year prior to the median date of last follow-up for the entire EuroSIDA cohort, which is a standard EuroSIDA measure of loss to follow-up[712].

Multiple imputations, with four sets of replication, were used to impute missing baseline data on HCV RNA, HCV genotype, HBsAg status and fibrosis staging. Only those that were positive for HCV RNA after imputation were included in the analysis. The following baseline covariates were included in the model:

- Age
- Gender
- Race
- Calendar year
- HIV transmission risk group
- Region of EuroSIDA (see Chapter 3 Section 3.1.1)
- Previous cardiovascular events (stroke, myocardial infarction, angina, endarterectomy, high blood pressure)
- Previous diabetes diagnosis
- HCV genotype
- CD4 cell count
- Nadir CD4 cell count
- HIV RNA
- HBsAg status (positive, negative)
- Minimum duration of HCV infection
- Liver fibrosis staging (Metavir F0/F1, F2/F3 or F4)

Minimum duration of HCV infection is the length of time prior to baseline that each individual had their first documented positive HCV antibody (HCVAb) or HCV RNA test result.

Stepwise variable selection was used to identify factors associated with progression to LRD. Covariates significant at the $P<0.1$ level were selected for entry into the model and those that remained significant at the $P<0.05$ level were selected to stay in the model. The LRD score was then calculated based on the estimated coefficients associated with the selected covariates included in the model. The coefficients were scaled so that the effect of not taking cART at baseline, compared with taking cART, represented a 1-unit increase in the LRD score. The other score components were then calculated by rounding each

coefficient to the nearest 0.5. An individual's LRD score is then calculated by summing all the LRD score components associated with their risk profile.

A univariable Fine and Gray Cox proportional hazards model with the LRD score as the only covariate and LRD as the endpoint was computed to describe the association between LRD and a 1-unit increase in the LRD score. Summary statistics were used to compare the LRD score between those that progressed to LRD and those that did not. The ability of the LRD score to correctly identify those who progressed to LRD was assessed using the area under the receiver-operating characteristics (ROC) curve and the c-statistic, in addition to calculating the sensitivity and specificity of the LRD score[687], and compared to an alternative score which only includes liver fibrosis staging.

The risk of progression to LRD was then categorised as low, medium-low, medium-high or high based on the quartiles of the LRD score distribution among those who progressed to LRD. Non-parametric cumulative incidence functions, which are not biased in the presence of competing risks, were then calculated to estimate the 5-year probability of LRD according to the categorised risk levels of the LRD score. The number of individuals needed to treat (NNTT) to prevent one LRD was then calculated for each LRD score risk category. The NNTT is derived as the inverse of the absolute risk reduction associated with a treatment[713]. The NNTT was calculated assuming a modest effect of treatment with DAAs (that on average treatment with DAA therapy would result in a 50% reduction of LRD) and an optimistic effect of treatment with DAAs (an 80% reduction of LRD).

Fine and Gray Cox proportional hazards models and cumulative incidence functions were estimated using the %PSHREG and %CIF validated SAS macros[663;664].

8.3.2.1 Validation of the LRD score in the Swiss HIV Cohort Study

In order to assess the prognostic ability of the LRD score outside of EuroSIDA, the score was externally validated using data provided by the Swiss HIV Cohort Study (SHCS). LRD score summary statistics, ROC curve and c-statistic, and non-parametric cumulative incidence functions were computed using the external SHCS data and compared with the results seen in the EuroSIDA cohort.

The SHCS is a prospective cohort study with ongoing enrolment of HIV-positive individuals[714]. Established in 1988 as a collaboration of Swiss universities, outpatient clinics, laboratories, smaller hospitals and private clinicians caring for HIV-positive individuals, the study remains representative of the Swiss HIV epidemic[714]. The SHCS currently includes data for 56% of all known HIV-positive individuals in Switzerland[714].

The SHCS study collects a large amount of data on patient demographics, results of HIV-specific tests (including CD4 cell counts, HIV RNA, liver biopsy results and liver transaminases), history of cART, and hepatitis-related data (including HCV antibody, HCV RNA and HBsAg) at 6-monthly intervals. At study entry and at each follow-up clinical visit a spectrum of laboratory tests belonging to the standard of care for HIV-positive individuals are also performed[714].

The SHCS has a well-established track record of excellent data quality, with quality control taking place at local sites by study nurses and again at the coordinating centre by qualified collaborators and computer programs when data are entered into the central database. Further, each time a follow-up visit is added to the database a graphical summary of key data is sent to the physician in charge of the individual for a supplementary round of quality control. More information on the SHCS can be found at www.shcs.ch.

In order to facilitate sharing of data between HIV cohort studies, in 2003 the SHCS together with the Copenhagen HIV Programme (CHIP) established the HIV Cohorts Data Exchange Protocol (HICDEP, www.hicdep.org)[715]. This data sharing platform has since been widely used in international HIV cohort collaborations and allows for consistent and accurate sharing of data. Upon establishment of collaborative interest in the LRD score analysis, the SHCS shared data on all their HIV/HCV coinfected individuals with EuroSIDA via the HICDEP format.

Although the SHCS is a contributor to the EuroSIDA study they also collect a large amount of data from individuals that are not included in EuroSIDA. For the purpose of validating the LRD score presented here, the validation SHCS cohort was restricted to those individuals that are not included in the EuroSIDA study.

8.4 Results

8.4.1 Generalizability and baseline characteristics

During the population selection process for this analysis 867/4,878 HCVAb positive individuals were excluded from the study as they did not have follow-up available after 1st January 2000 (see Figure 8.1). To consider the generalizability of the results in this chapter I used multivariable logistic regression, adjusted for the covariates listed above, to assess how these excluded individuals differed from those that were included in the final analysis population.

Those without follow-up after 1st January 2000 and therefore excluded from the analysis, were less likely to reside in Western Europe (adjusted odds ratio (aOR): 0.62 (95% CI 0.45 – 0.84; *P*=0.0013)) and more likely to reside in Eastern Europe (aOR: 1.62 (1.11 – 2.38; *P*=0.0013)), compared with Southern Europe. They also had lower CD4 cell counts at baseline (aOR: 0.77 (0.69 – 0.86; *P*<0.0001) per doubling) but higher nadir CD4 cell counts (aOR: 1.17 (1.08 – 1.28; *P*=0.0003) per doubling). In addition, they had longer minimum HCV infection durations (aOR: 1.09 (1.05 – 1.13; *P*<0.0001) per year) and were more likely to have HCV RNA data available (aOR: 1.85 (1.48 – 2.32; *P*<0.0001)), however, they were substantially more likely to have no data on liver fibrosis (aOR: 82.5 (57.7 – 118.1; *P*<0.0001)), compared to those with follow-up after 1st January 2000.

In the total study population of 4,011 HIV/HCV coinfected individuals, 787 deaths from any cause were recorded during a total of 17,389 person years follow-up (PYFU) (median 3.3 years per person (inter-quartile range (IQR) 1.5 – 6.7)) to June 2014. The overall incidence of all-cause mortality was 45.3 (95% CI 42.2 – 48.3) per 1,000 PYFU. LRD accounted for 180/787 (22.9%) of all recorded deaths, giving an incidence of LRD of 10.4 (8.8 – 11.9) per 1,000 PYFU.

Baseline characteristics of the 4,011 HIV/HCV coinfected individuals included in this analysis are shown in Table 8.3, stratified by study endpoint. The study population was mostly white (93.0%) males (68.2%) with a median age of 37 (IQR 31 - 43). The largest contributing regions of EuroSIDA were Eastern Europe (31.0%) and Southern Europe (25.0%), although all European regions were well represented. The predominant mode of HIV transmission reported was via injecting drug use (IDU) (69.2%), followed by heterosexual contact (15.3%).

Table 8.3 Baseline Characteristics of all HIV/HCV coinfected individuals included in the analysis stratified by endpoint

Median (IQR) %		*All (N=4011)*	*Endpoint*			*P-Value**
			LRD (N=180)	*Non-LRD (N=607)*	*Censored/Alive (N=3224)*	
Age		37 (31 - 43)	40 (36 - 45)	39 (33 - 45)	37 (30 - 43)	<.0001
Male		2734 (68.2)	126 (70.0)	448 (73.8)	2160 (67.0)	0.0037
White		3732 (93.0)	167 (92.8)	563 (92.8)	3002 (93.1)	0.94
Region of EuroSIDA	South	1004 (25.0)	55 (30.6)	107 (17.6)	842 (26.1)	<.0001
	West Central	564 (14.1)	38 (21.1)	92 (15.2)	434 (13.5)	
	North	519 (12.9)	44 (24.4)	151 (24.9)	324 (10.0)	
	East Central	578 (14.4)	13 (7.2)	56 (9.2)	509 (15.8)	
	East	1243 (31.0)	27 (15.0)	187 (30.8)	1029 (31.9)	
	Argentina	103 (2.6)	3 (1.7)	14 (2.3)	86 (2.7)	
HIV transmission route	MSM	372 (9.3)	13 (7.2)	35 (5.8)	324 (10.0)	<.0001
	IDU	2774 (69.2)	136 (75.6)	478 (78.7)	2160 (67.0)	
	Heterosexual	612 (15.3)	17 (9.4)	65 (10.7)	530 (16.4)	
	Other	253 (6.3)	14 (7.8)	29 (4.8)	210 (6.5)	
HCV-RNA status	Negative	254 (6.3)	5 (2.8)	38 (6.3)	211 (6.5)	0.010
	Positive	2459 (61.3)	131 (72.8)	356 (58.6)	1972 (61.2)	
	Unknown	1298 (32.4)	44 (24.4)	213 (35.1)	1041 (32.3)	
Duration of HCV infection	Years	2.4 (0.4 - 5.4)	4.0 (1.8 - 7.7)	2.9 (0.7 - 6.0)	2.2 (0.3 - 5.1)	<.0001
HCV genotype	G1	997 (24.9)	65 (36.1)	138 (22.7)	794 (24.6)	0.0001
	G2	54 (1.3)	4 (2.2)	13 (2.1)	37 (1.1)	

Median (IQR) %		*All (N=4011)*	*Endpoint*			*P-Value**
			LRD (N=180)	*Non-LRD (N=607)*	*Censored/Alive (N=3224)*	
	G3	578 (14.4)	27 (15.0)	97 (16.0)	454 (14.1)	
	G4	281 (7.0)	15 (8.3)	26 (4.3)	240 (7.4)	
	Unknown	2101 (52.4)	69 (38.3)	333 (54.9)	1699 (52.7)	
HBsAg status	Negative	3199 (79.8)	139 (77.2)	472 (77.8)	2588 (80.3)	0.0002
	Positive	276 (6.9)	26 (14.4)	47 (7.7)	203 (6.3)	
	Unknown	536 (13.4)	15 (8.3)	88 (14.5)	433 (13.4)	
CD4 cell count	Cell/mm^3	385 (240 - 569)	232 (120 - 430)	270 (140 - 465)	411 (272 - 589)	<.0001
CD4 nadir	Cell/mm^3	163 (67 - 285)	103 (37 - 200)	110 (47 - 212)	177 (78 - 297)	<.0001
HIV RNA	<400copies/ml	2155 (60.3)	81 (48.2)	228 (44.4)	1846 (63.9)	<.0001
Liver fibrosis	F0/F1	2624 (65.4)	38 (21.1)	275 (45.3)	2311 (71.7)	<.0001
	F2/F3	225 (5.6)	21 (11.7)	31 (5.1)	173 (5.4)	
	F4	328 (8.2)	56 (31.1)	48 (7.9)	224 (6.9)	
	Unknown	834 (20.8)	65 (36.1)	253 (41.7)	516 (16.0)	

***P-value from Kruskal-Wallis or Chi-square test comparing the different study endpoints**

LRD: Liver-related death; MSM: Men who have sex with men; IDU: Injecting drug users; HBsAg: Hepatitis B surface antigen

At baseline 61.3% of those included in the study population were positive for HCV RNA, 6.3% were negative while 32.4% had no data available on HCV RNA. The median minimum duration of HCV infection was 2.4 (IQR 0.4 – 5.4) years. The most frequent HCV genotype was G1 (24.9%), followed by G3 (14.4%), G4 (7.0%) and G2 (1.3%); for 52.4% HCV genotype was not known at baseline. The majority of participants in this study were negative for HBsAg at baseline (79.8%), 6.9% were positive, while 13.4% had no data available for HBsAg.

At baseline data on liver fibrosis were available for 79.2% of the study population. Overall 65.4% had F0/F1 fibrosis while 5.6% and 8.2% had F2/F3 and F4 fibrosis, respectively. Interestingly, among those who died of LRD 31.1% had F4 fibrosis at baseline and 21.1% had F0/F1 fibrosis. In comparison, 45.3% those who died from non-LRD had F0/F1 fibrosis at baseline and 7.9% had F4 fibrosis (*P*<0.0001).

The overall median baseline CD4 cell count of those included in the study was 385 cells/mm^3 (IQR 240 - 569), however, there were large discrepancies between those who were censored at the end of follow-up (411 (272 - 589)) and those who died of LRD (232 (120 - 430)) or non-LRD (270 (140 - 465)) (*P*<0.0001). Similarly, those who were censored at the end of follow-up also had higher nadir CD4 cell counts (177 (78 - 297)) compared with those who died of LRD (103 (37 - 200)) or non-LRD (110 (47 - 212)) (*P*<0.0001). Further, the proportion of those included in the analysis who had baseline HIV viral load <400 copies/ml was higher in those censored at the end of follow-up (63.9%) compared to those who died of LRD (48.2%) or non-LRD (44.4%) (*P*<0.0001).

8.4.2 Derivation of the LRD score

The stepwise variable selection process used in the Fine and Gray competing risks Cox proportional hazards model selected the covariates shown in Table 8.4. Similar to the analysis shown in Chapter 7, age, CD4 cell count, liver fibrosis levels, HBV coinfection, minimum duration of HCV infection and whether taking cART or not at baseline were all significantly associated with progression to LRD. As expected the largest contributing factors to the LRD score were low CD4 cell counts (<50cells/mm^3 scores 3.5, while 50-100cells/mm^3 scores 3) and significant levels of liver fibrosis (F2/F3 fibrosis scores 3, while F4 fibrosis scores 4.5).

Table 8.4 Factors associated with progression to LRD and their contributions to the LRD score

Covariate		*β*	*P-Value*	*Scaled β*	*Score contribution*
Age >35	Vs. ≤35	0.93	<0.0001	2.50	2.5
CD4 <50	Vs. ≥500cells/mm^3	1.24	0.0003	3.34	3.5
50≤ CD4 <100		1.18	0.0002	3.19	3
100≤ CD4 <300		0.41	0.074	1.09	1
300≤ CD4 <500		0.03	0.89	0.09	0
F2/F3	Vs. F0/F1 Fibrosis	1.06	<0.0001	2.84	3
F4		1.71	<0.0001	4.58	4.5
HBsAg Positive	Vs. HBsAg Negative	0.66	0.0052	1.76	2
2≤ HCV Dur* <10	Vs. HCV Dur* <2	0.60	0.0014	1.60	1.5
10≤ HCV Dur*		0.87	0.0009	2.32	2.5
Off cART	Vs. Taking cART	0.37	0.020	1	1

β: Estimated coefficient from the model; *HCV Dur: Minimum HCV infection duration

Table 8.5 LRD score calculation sheet

Variable	*Criteria*	*Score contribution*	*Score*
Age	< 35	0	
	≥ 35	2.5	2.5
CD4 cell count	0 ≤ cells/mm^3 < 50	3.5	
	50 ≤ cells/mm^3 < 100	3	
	100 ≤ cells/mm^3 < 300	1	1
	300 ≤ cells/mm^3 < 500	0	
	500 ≤ cells/mm^3	0	
Taking antiretroviral therapy	Yes	0	
	No	1	1
HBV status	HBsAg positive	2	
	HBsAg negative	0	0
Duration of HCV infection	< 2 years	0	
	2 ≤ years < 10	1.5	1.5
	10 ≤ years	2.5	
Fibrosis staging	F0/F1	0	
	F2/F3	3	3
	F4	4.5	
Total		=	9

An illustration of a LRD score calculation is shown in Table 8.5 for a 45-year-old individual with a CD4 cell count of 250cells/mm^3, not currently taking cART, with no evidence of HBV coinfection, a minimum duration of HCV infection of 4 years, and F2 liver fibrosis. Summing all the component parts of the LRD score this individual has a LRD score of 9.

8.4.3 LRD score summary statistics and distribution in EuroSIDA

The overall mean LRD score for the whole study population was 4.3 (95% CI 4.2 – 4.3), while the median was 4 (IQR 2.5 – 5.5). As the mean and median LRD scores for the whole study population are close together it indicates that the score is not skewed in this population. Figure 8.2 shows the distribution of LRD scores among those who progressed to LRD and those who did not. Both distributions appear to be approximately normally distributed, however, LRD scores among those who progressed to LRD were higher than those for individuals that did not progress to LRD. The mean LRD score was significantly higher among those who progressed to LRD (7.3 (95% CI 6.9 – 7.8) vs. 4.1 (4.0 – 4.2); *P*<0.0001) compared to those that did not progress to LRD. The median LRD score was also significantly higher among those who progressed to LRD (7.25 (4.75 – 9.5) vs. 4 (IQR 4.0 – 5.5); *P*<0.0001) and in each distribution the mean and median were approximately the same.

In a univariable Fine and Gray model including the LRD score as the only covariate, a 1-unit increase in the LRD score was associated with 1.4-fold increased risk of LRD (sub-distribution hazard ratio (sHR): 1.44 (95% CI 1.37 – 1.51; *P*<0.0001). The LRD score achieved a c-statistic of 0.81 (95% CI 0.77 – 0.85), which indicates strong differentiation between those who progress to LRD and those that do not (Figure 8.3). The LRD score performed substantially better than an alternative score which only included liver fibrosis staging, which achieved a c-statistic of 0.72 (95% CI 0.68 – 0.76). In formal comparison the difference between the performance of the two scores was highly statistically significant (*P*<0.0001).

A LRD score cut-off of 5.5 provided the highest combination of sensitivity and specificity over 5-years of follow-up. Using a cut-off of 5.5 the LRD score achieved a 5-year sensitivity of 68.0% and a specificity of 77.5%, which means that 68% of those with scores above 5.5 experience LRD, while 77.5% of those with scores below 5.5 do not experience LRD.

Figure 8.2 LRD score distribution among those who did and did not progress to LRD

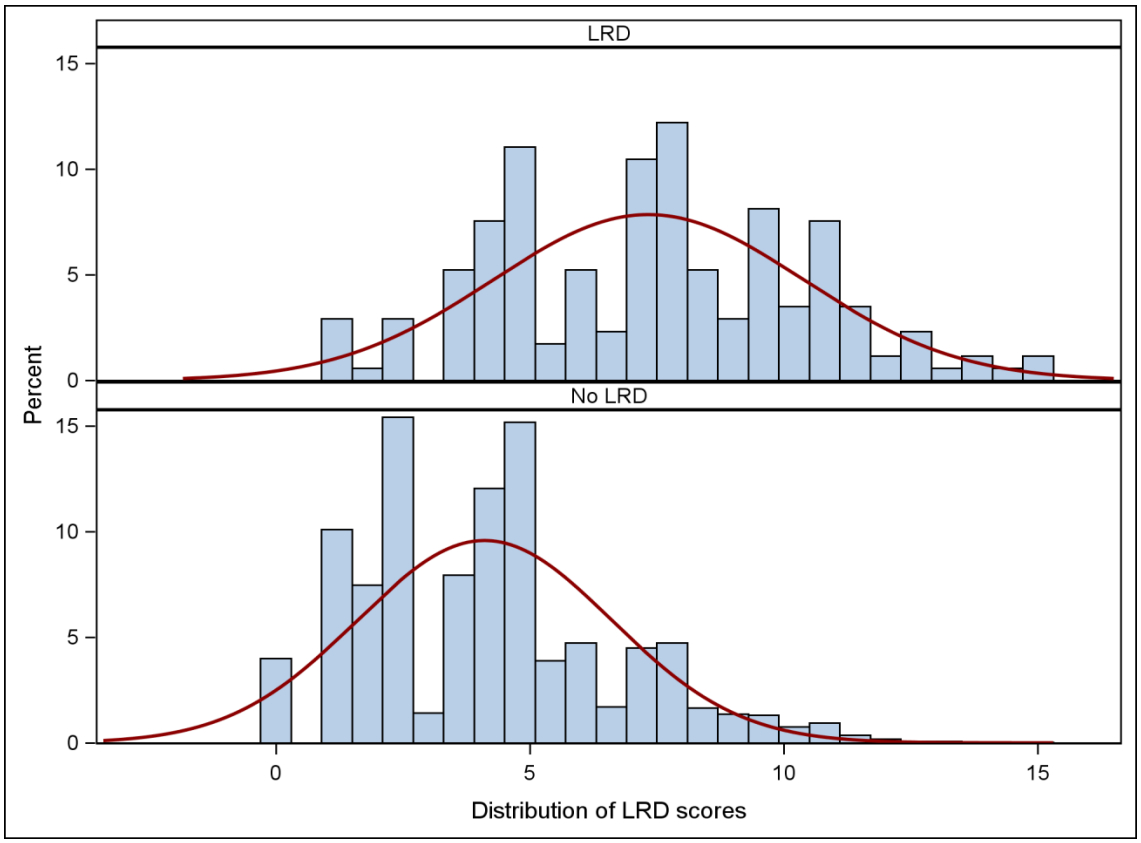
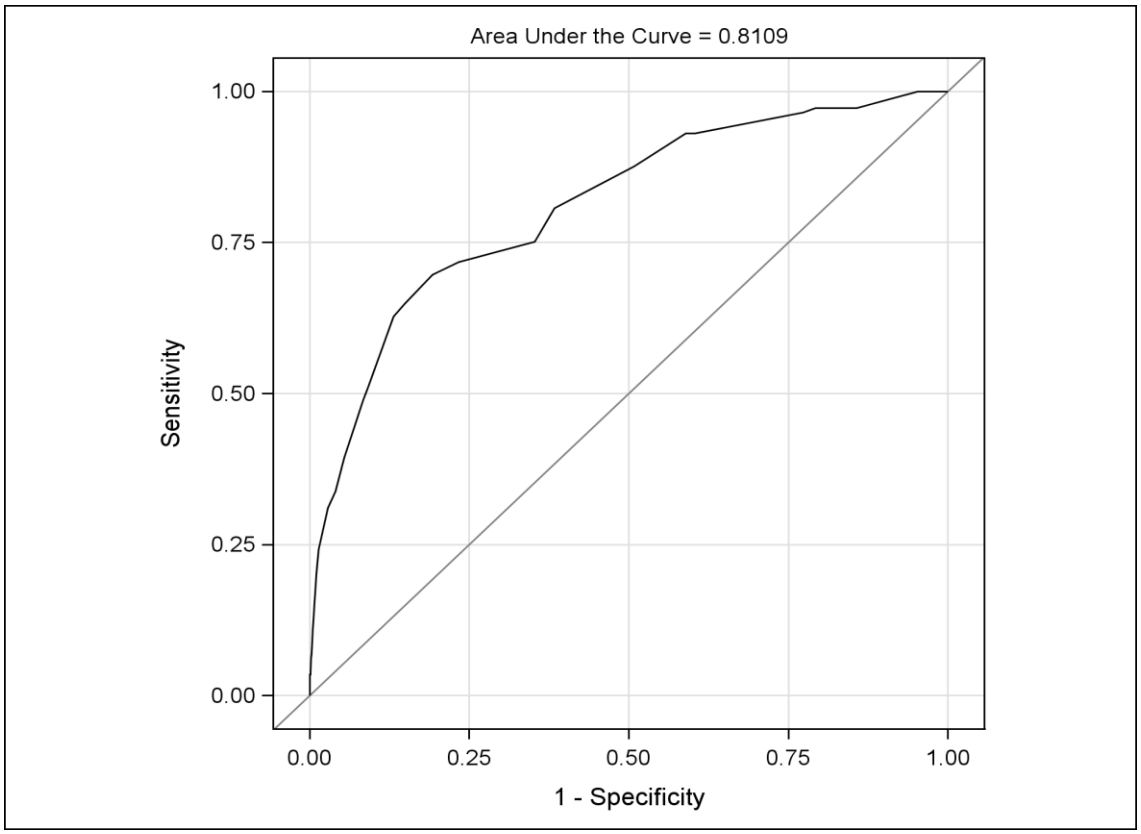


Figure 8.3 LRD score ROC curve



The quartiles of the distribution of LRD scores among those who progressed to LRD were used to categorise individuals into low, medium-low, medium-high and high risk of progression to LRD, shown in Table 8.6. Therefore, the individual in the example given in Table 8.5 would be considered to have medium-high risk of progression to LRD.

Table 8.6 LRD score risk categories

LRD score risk category	***LRD score range***
Low	LRD score <4.75
Medium-low	4.75≤ LRD score <7.25
Medium-high	7.25≤ LRD score <9.5
High	LRD score ≥9.5

8.4.4 Incidence and cumulative incidence of LRD by LRD score risk category

The overall incidence of LRD and the NNTT to prevent one LRD are shown in Table 8.7, stratified by LRD score risk category. The incidence of LRD was low among those in the low risk category (0.44 per 100 PYFU (95% CI 0.31 – 0.58)), but increased steadily in those at medium-low, medium-high and high risk (1.01 (0.71 – 1.31), 3.05 (2.05 – 4.04) and 8.33 (6.14 – 10.52) per 100 PYFU, respectively).

Assuming that treatment with DAA therapy will lead to a 50% reduction in the overall incidence of LRD, then 102 (95% C.I. 74 – 144) coinfected individuals in the low LRD score risk category would need to be treated to prevent one LRD over a period of 5-years. However, the NNTT falls to 45 (33 - 64), 18 (13 - 27) and 8 (6 - 10) for those in the medium-low, medium-high and high risk categories, respectively. Assuming a more optimistic outcome of DAA therapy, that treatment will lead to an 80% reduction in the overall incidence of LRD, then 58 (42 - 82) coinfected individuals in the low LRD score risk category would need to be treated to prevent one LRD over a period of 5-years, falling to 26 (19 - 36), 10 (8 - 15) and just 4 (4 - 6) for those in the medium-low, medium-high and high risk categories, respectively.

Cumulative incidence functions for progression to LRD are shown in Figure 8.4, stratified by LRD score risk category. The LRD score risk categories were able to accurately identify those at the highest and lowest risk of progression to LRD, with highly significant separation between strata (P<0.0001). The 5-year probability of LRD

Table 8.7 Incidence of LRD and number needed to treat to prevent one LRD by LRD score risk category

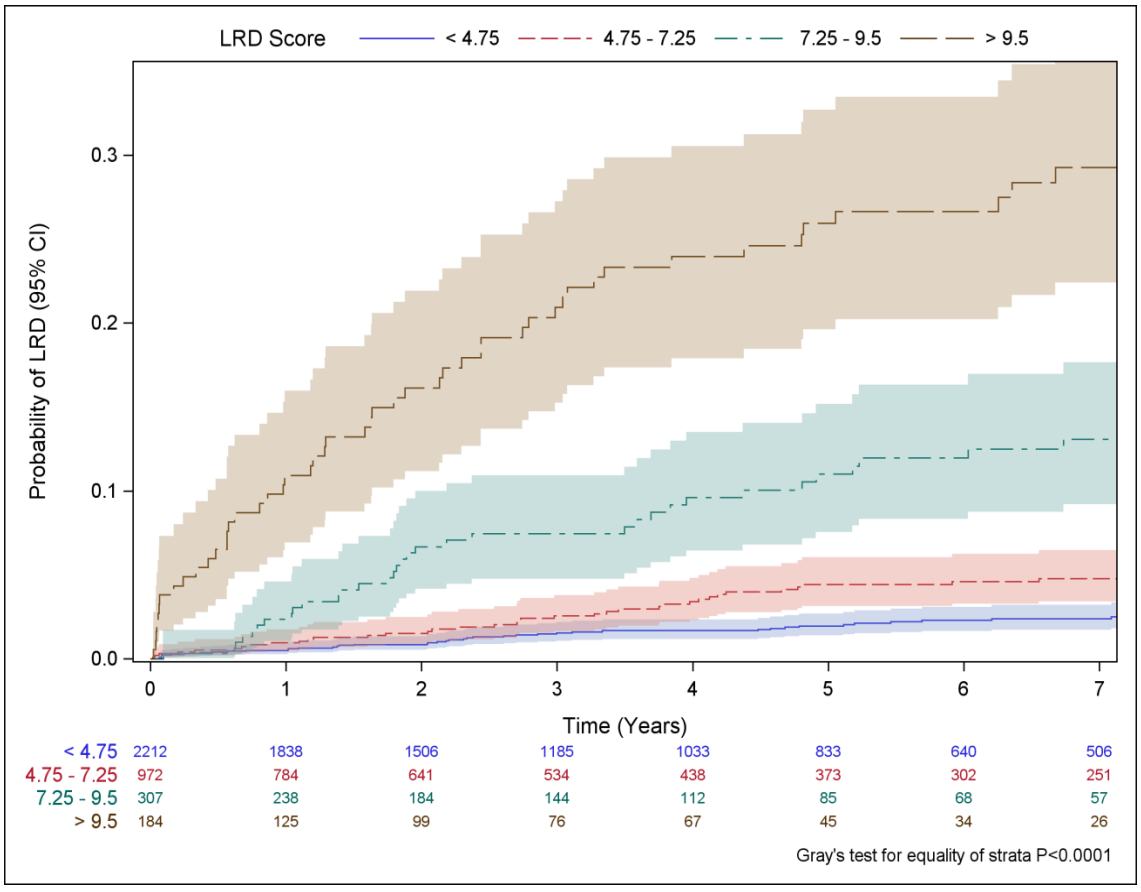
LRD score category	*Incidence of LRD/100 PYFU (95% CI)*	*NNTT 50% (95% CI)*	*NNTT 80% (95% CI)*
Low	0.44 (0.31 – 0.58)	102 (74 – 144)	58 (42 - 82)
Medium-low	1.01 (0.71 – 1.31)	45 (33 - 64)	26 (19 - 36)
Medium-high	3.05 (2.05 – 4.04)	18 (13 - 27)	10 (8 - 15)
High	8.33 (6.14 – 10.52)	8 (6 - 10)	4 (4 - 6)

NNTT: Calculated based on the 5-year incidence of LRD

NNTT 50%: Number needed to treat to prevent one LRD assuming that treatment with DAAs leads to an average 50% reduction in LRD.

varied widely across the score groups illustrating the good discriminatory properties of the score. The 5-year probability of LRD was 2.0% (95% CI 1.4 – 2.7) among those in the low risk category, increasing to 4.4% (3.1 – 6.0) in those at medium-low risk, 11.0% (7.6 – 15.2) in those at medium-high risk, and as high as 25.9% (19.6 – 32.7) among those in the high risk category.

Figure 8.4 Cumulative incidence of LRD by LRD score risk category



Interestingly, differentiation between the low (0.51% (95% CI 0.27 – 0.89)), medium-low (0.95% (0.47 – 1.75)) and medium-high (2.36% (1.05 – 4.60)) risk categories was less pronounced for the 1-year probability of LRD. However, those in the high risk category had a substantially higher 1-year probability of LRD (10.9% (6.9 – 16.0)), indicating the high risk of LRD these individuals face in the short and medium term.

8.4.5 Validation of the LRD in the Swiss HIV Cohort Study

In the SHCS population of 1,303 HIV/HCV coinfected individuals there were 157 deaths from any cause recorded in a total of 7,742 PYFU to June 2014. The overall incidence of all-cause mortality was 20.3 per 1,000 PYFU (95% CI 17.1 – 23.4). LRD accounted for 38/157 (24.2%) of all recorded deaths, giving an incidence of LRD of 4.9 per 1,000 PYFU (3.4 – 6.5). A brief description of the data provided by the SHCS is given in Table 8.8, with EuroSIDA data given for comparison.

Similar to the EuroSIDA population, the SHCS population was mostly male (66.5%) with a median age of 42 (IQR 37 - 46). The most common mode of HIV transmission was IDU (47.0%) followed by heterosexual contact (16.6%) and MSM (12.5%). Although IDU was the most common mode of HIV transmission the proportion of IDUs in the whole study population was substantially lower than in EuroSIDA (47.0% vs. 69.2%; P<0.0001). HBsAg was detected among 6.8% of the SHCS population and the median minimum duration of HCV infection was 7.1 years, substantially higher than in EuroSIDA (7.1 (IQR 4.0 – 10.3) vs. 2.4 (0.4 – 5.4); P<0.0001).

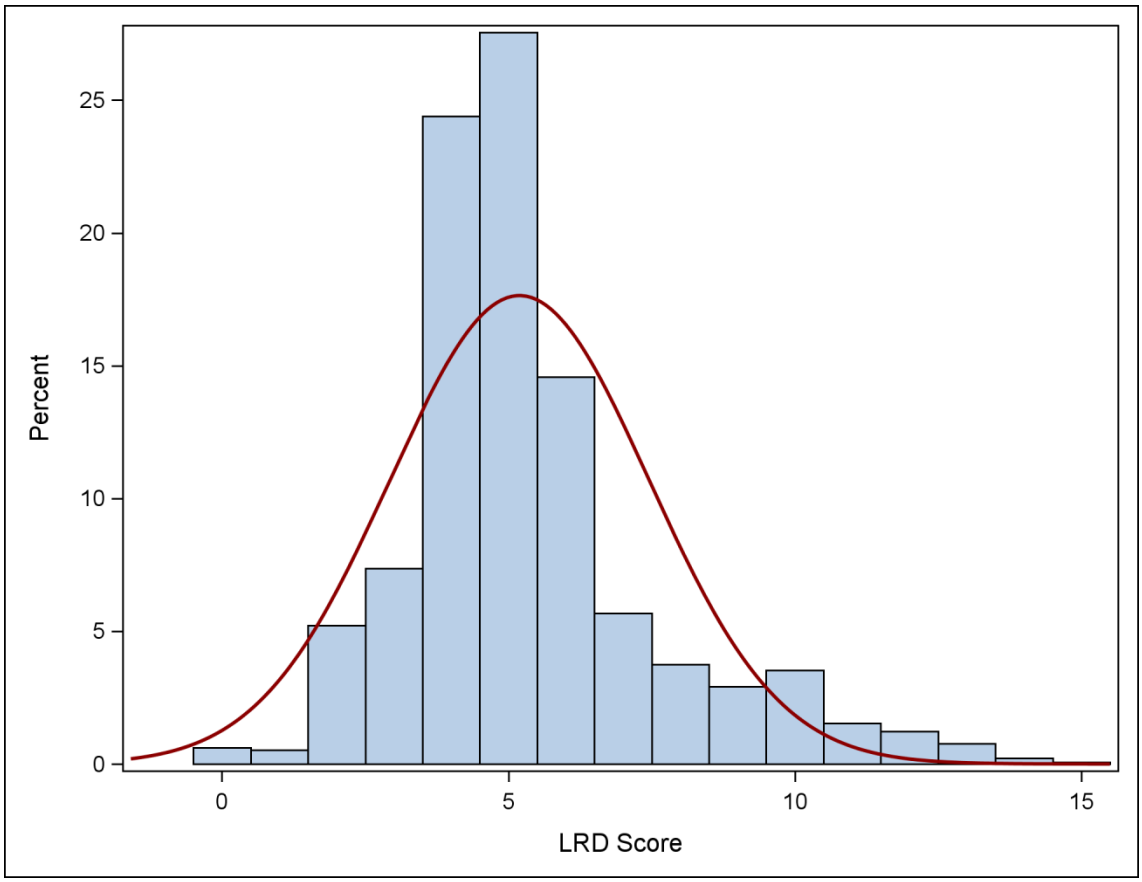
The median baseline CD4 cell count of the SHCS population was 402 (IQR 365 - 588), while the median baseline nadir CD4 cell count was almost identical to that of EuroSIDA (165 (70 - 282) vs. 163 (67 - 285)). Data on liver fibrosis levels were complete at baseline in the SHCS. The proportion of individuals reporting F2/F3 fibrosis or F4 fibrosis was comparable between the SHCS and EuroSIDA (6.3% vs. 5.6% and 10.4% vs. 8.2%, respectively).

The distribution of LRD scores in the SHCS is shown in Figure 8.5. The distribution of LRD scores appears to be approximately normal, as evidenced by the mean (5.2 (95% CI 5.1 – 5.3)) and median (5 (IQR 4 - 6)) being approximately the same. The mean LRD score was significantly higher among those who progressed to LRD (7.5 (95% CI 6.5 – 8.4) vs. 5.1 (5.0 – 5.2); P<0.0001) compared with those that did not progress to LRD, and very similar to the mean LRD score in EuroSIDA among those who progressed to LRD (7.3 (6.9 – 7.8)).

Table 8.8 Baseline characteristics of HIV/HCV coinfected individuals from the Swiss HIV Cohort study

Variable		*SHCS*	*EuroSIDA*
HCV RNA positive coinfected individuals (Total PYFU)		1303 (7,742)	4011 (17,389)
Liver-related deaths (Total deaths)		38 (157)	180 (787)
Baseline	Median (IQR)	Dec 05 (Dec 05 – Jul 07)	Nov 05 (Sep 01 – Nov 08)
Age	Median (IQR)	42 (37 – 46)	37 (31 - 43)
Male	N (%)	866 (66.5)	2734 (68.2)
Injecting drug user	N (%)	612 (47.0)	2774 (69.2)
MSM	N (%)	163 (12.5)	372 (9.3)
Heterosexual	N (%)	216 (16.6)	612 (15.3)
HBsAg positive	N (%)	89 (6.8)	276 (6.9)
Minimum duration HCV positive (Years)	Median (IQR)	7.1 (4.0 – 10.3)	2.4 (0.4 – 5.4)
Taking cART	N (%)	925 (71.0)	2640 (65.8)
CD4 cell count (cells/mm^3)	Median (IQR)	402 (365 – 588)	385 (240 - 569)
Nadir CD4 cell count (cells/mm^3)	Median (IQR)	165 (70 – 282)	163 (67 – 285)
Fibrosis staging (N (%))	F0/F1	1086 (83.4)	2624 (65.4)
	F2/F3	82 (6.3)	225 (5.6)
	F4	135 (10.4)	328 (8.2)
	Unknown	0	834 (20.8)

Figure 8.5 Distribution of LRD scores in the Swiss HIV Cohort study



The LRD score achieved a c-statistic of 0.79 (95% CI 0.70 – 0.88) in the SHCS (Figure 8.6), which is very similar to the c-statistic of 0.81 (95% CI 0.77 – 0.85) seen in the EuroSIDA study population. A c-statistic of 0.79 indicates reasonable to strong differentiation between those that do and do not progress to LRD. More importantly, the LRD score appears to have a similar prognostic capability for progression to LRD in the SHCS as it displayed in EuroSIDA.

Cumulative incidence functions for progression to LRD in the SHCS stratified by the LRD risk categories are shown in Figure 8.7. The LRD score risk categories were able to accurately identify those at the highest and lowest risk of progression to LRD, with highly significant separation between strata (P<0.0001). The 5-year probability of LRD was 0.5% (95% CI 0.1 – 1.6) among those in the low risk category, but increased to 2.4% (1.3 – 4.2), 8.1% (3.2 – 15.8) and 11.6% (5.6 – 19.9) among those at medium-low, medium-high and high risk of progression to LRD, respectively.

Figure 8.6 LRD score ROC curve in the Swiss HIV Cohort study

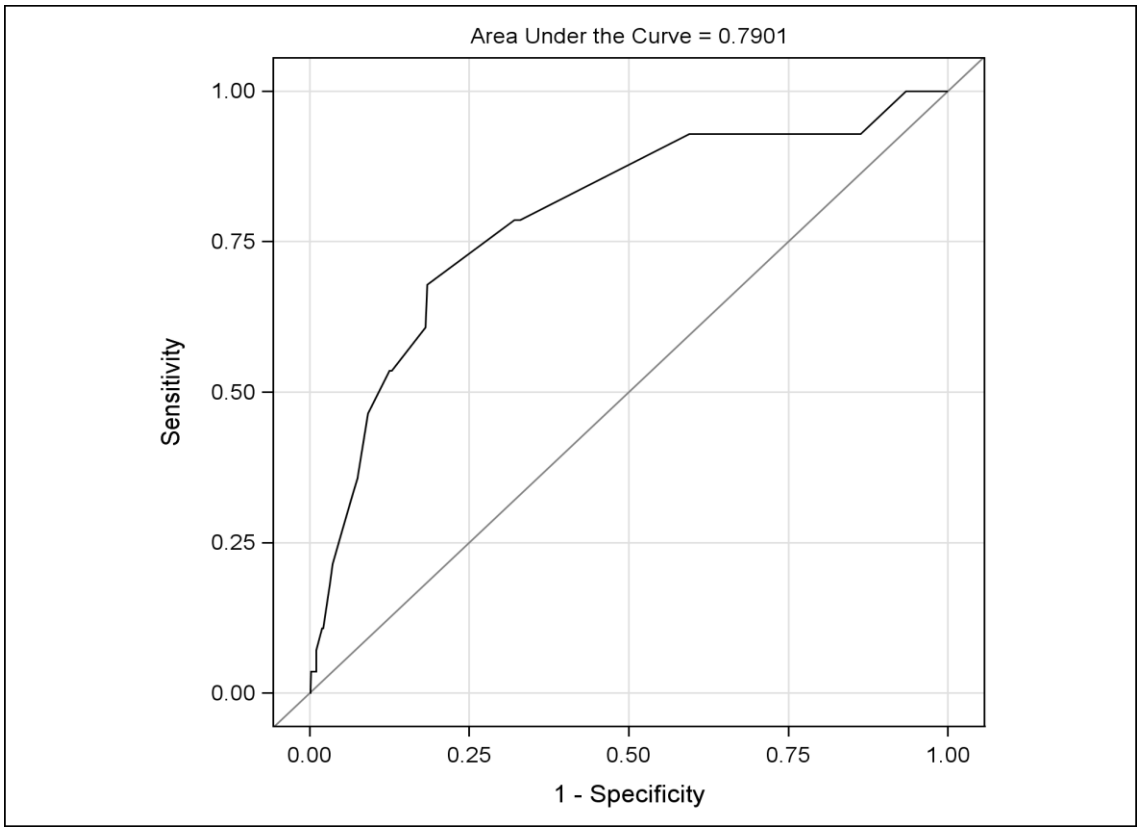
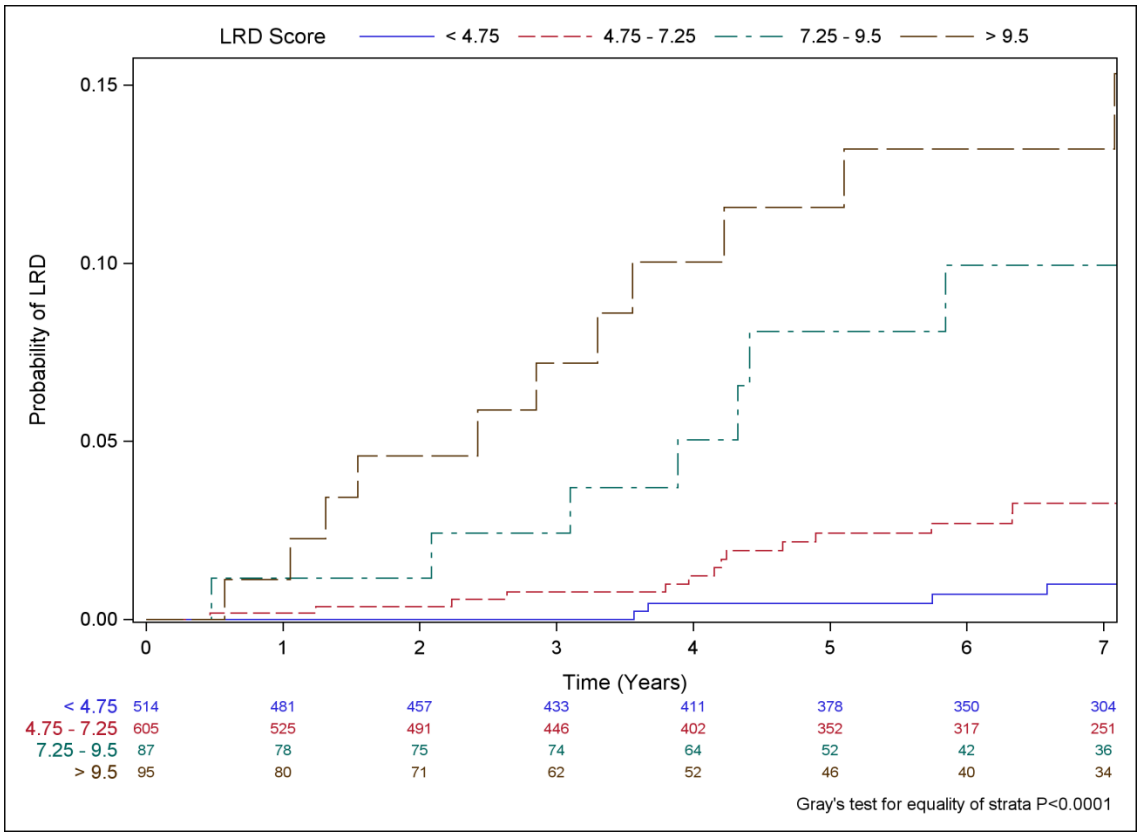


Figure 8.7 Cumulative incidence of LRD by LRD score risk category in the Swiss HIV Cohort study



8.5 Discussion

Performance of the LRD score

This chapter builds on the analysis presented in the previous chapter, describing factors associated with progression to LRD, to construct a single, easily applicable prognostic score for progression to LRD among HIV/HCV coinfected individuals. The LRD score was developed using data from the EuroSIDA study and validated on external SHCS data. To my knowledge, this is the first prognostic score developed with the specific intention of identifying HIV/HCV coinfected individuals at greatest risk of progression to LRD in the presence of HIV-related competing risks. Used in the clinical setting the LRD score may help health care providers make difficult decisions about who should be prioritised for treatment with new expensive DAA therapy for treatment of HCV. Coinfected individuals in the high and medium-high should be prioritised for treatment as they are at the greatest risk of LRD.

The LRD score was able to accurately differentiate between those at high and low risk of LRD in the EuroSIDA derivation cohort and showed excellent repeatability in the SHCS validation cohort. Although no direct comparison is available, as this is the first score developed among HIV/HCV coinfected individuals with respect to LRD, the LRD score performs well in comparison to other commonly used scores. The well-known Framingham risk score commonly used in clinical practise to predict cardiovascular disease achieved c-statistics of 0.73 and 0.71 in men and women, respectively[686]. The VACS Index, which predicts all-cause mortality after 1-year of cART for HIV-positive individuals, was considered to achieve strong differentiation in its derivation cohort (c-statistic: 0.78) and validation cohort (c-statistic: 0.82) and the LRD score achieves almost identical levels of differentiation in this study[688].

Interestingly, the VACS Index study showed that using information on multiple organ systems significantly improved prediction of 1-year all-cause mortality, specifically mentioning the addition the liver fibrosis marker FIB-4[688]. The LRD score includes the APRI liver fibrosis marker, which is one of the main contributors to the score calculation. It is possible that the additional benefit of including FIB-4 in the VACS Index was attributable to a better prediction of progression to LRD, although it is not possible to say definitively as the VACS study differentiates deaths as HIV-related and non-HIV-related rather than liver-related and non-liver-related[688].

Fibrosis staging, as measured by the APRI score, was the largest contributor to the LRD score calculation. However, other HIV-related factors such as CD4 cell count were also

heavily weighted. Comparing the LRD score to an alternative score, which only included the liver fibrosis component, showed that prediction based solely on fibrosis staging was highly significantly inferior to the full LRD score.

The Child-Pugh and MELD scores, developed to predict mortality among cirrhotic patients, attempt to describe the function of the liver through a number of continuous and quantitative measures[692;700]. The LRD score was developed with these scores in mind. The Child-Pugh score is often criticised because the components were chosen empirically and all weighted equally while the MELD score is not considered to be applicable in the clinical setting as the equation is not easily interpreted[693]. The LRD score was developed by selecting a large number of variables known to be associated with LRD from previous EuroSIDA work and empirical knowledge. The most important of these variables were then selected by the model so that each component has a weighted and independent effect on LRD. Further, with the use of the calculation sheet the score can be easily calculated in everyday practice to determine an individual's risk category.

The LRD score also performs well in comparison to these historically useful scores. The Child-Pugh and MELD scores have traditionally been associated with reasonable to strong differentiation between patients with respect to survival following TIPS, development of cirrhosis, and development of liver disease (c-statistics ranging between 0.65 and 0.85)[702-705]. In comparison, the LRD score shows strong differentiation among HIV/HCV-coinfected individuals with respect to progression to LRD (c-statistics: 0.81 and 0.79 in the derivation and validations cohorts), highlighting the potential usefulness of the LRD score in the clinical setting.

This analysis aimed to determine whether the factors identified in Chapter 7 could be combined into a prognostic score to predict progression to LRD. The results presented here clearly demonstrate that the LRD score has a strong ability to differentiate between those at the highest and lowest risk of LRD. This analysis was also specifically designed to make the LRD score easy to calculate and interpret. Therefore, individual risk profiles are collected into risk categories. While this strategy may be useful in the clinical setting to determine those at the greatest risk of LRD, the optimal use of the LRD score would allow for an individualised 5-year risk of LRD. However, this would require individual characteristics to be input into the underlying formula from the statistical model and may not be easily interpretable in the clinical setting.

With this in mind, the logical next step is to make an individualised version of the LRD score widely available, while maintaining the ease of use and interpretation. One potential

way to achieve this aim would be to develop a mobile phone application or link to the EuroSIDA webpage, where individuals could input their characteristics and receive an individualised 5-year risk of LRD. These ideas are already being discussed and I foresee work progressing on whichever method is easier to administer progressing in the near future.

Mortality rates in EuroSIDA and the SHCS

While life expectancy in HIV-monoinfected individuals with high CD4 cell counts has started to approach that of the general population[8;716], mortality rates among HIV/HCV coinfected individuals remain stubbornly high. In 2007 *Lohse et al* reported that between 1995 and 2005 in Denmark the mean survival time after age 25 for HIV-monoinfected individuals was double that of HIV/HCV-coinfected individuals (39 vs. 20 years)[656]. Differences between individual characteristics of these two groups are often cited as reasons for the higher mortality seen for coinfected individuals. Coinfected individuals are typically more likely to be part of difficult to treat groups participating in active IDU and alcohol abuse. In this study 69% of the coinfected individuals included reported IDU as the route of HIV transmission. However, interplay between the HIV and HCV viruses is also a contributing factor to increased mortality in coinfected individuals. Liver fibrosis progression is known to be accelerated in the presence of low CD4 cell counts and the LRD score shows that these two factors should be considered the most important determinants of progression to LRD[414;671].

A cut-off point of 5.5 provided the optimal combination of sensitivity and specificity for the LRD score. However, the strength of the LRD score appears to come from the excellent differentiation of risk categories for progression to LRD. In the EuroSIDA cohort those in the low risk category had a negligible 5-year probability of LRD, whereas those in the medium-high and high risk categories had a significant 5-year probability of progression to LRD, as high as 26% for the high risk group. Similar patterns were observed in the SHCS cohort with negligible risk for those in the low risk category and substantial risk of progression to LRD in those with the high risk categories.

Although the risk categories continued to show excellent differentiation in the SHCS it is important to note that those in the high risk category were approximately half as likely to progress to LRD in 5-years as those in EuroSIDA (12% vs. 26%). The lower probability of progression to LRD in the SHCS reflects the lower overall incidence of LRD reported in the study. In EuroSIDA the incidence of LRD was approximately double that of the SHCS (10.4 vs. 4.9 per 1,000 PYFU) over the study period. However, this shows that the LRD score can perform well in different risk settings, although the overall incidence of the LRD was

lower in the SHCS the risk score categories were still able to accurately identify those at the highest and lowest risk of progression to LRD.

Competing risks and the influence of individual factors on who to treat

The LRD score was developed specifically to predict progression to LRD among HIV/HCV-coinfected individuals in the presence of HIV-related competing risks. Therefore, the LRD score is ideally placed to help clinicians with difficult decisions regarding which HIV/HCV-coinfected individuals should be prioritised for new expensive DAA treatments for HCV. In the resource-limited setting studies of HCV-monoinfection have shown that the most cost effective strategies involve restricting treatment to those with higher levels of liver fibrosis[709;710]. In HIV/HCV-coinfection decisions on who to treat become somewhat more complicated by interplay between HIV- and HCV-related risk factors.

The LRD score can identify those at the greatest risk of progression to LRD but decisions about who to treat must then recognise the leading contributors to a high LRD score. For example, an individual with a high risk LRD score driven by a high degree of liver fibrosis, concurrent HBV infection and a long duration of HCV infection, but not a low CD4 cell count, would be a leading candidate for DAA therapy. In this instance DAA therapy if successful, along with a cART regimen containing drugs active against HBV, could remove the driving force behind progression of liver fibrosis and lower the risk of progression to LRD.

An individual with a high risk LRD score driven by a low CD4 cell count, concurrent HBV infection and a high degree of liver fibrosis should also be a leading candidate for DAA therapy[416]. However, the low CD4 cell count should also be addressed by ensuring they are taking appropriate cART, including drugs active against HBV. In this instance it may be preferable to delay treatment with DAAs until the CD4 cell count has increased as the low CD4 cell count is a possible contributor to fibrosis progression as well as a strong risk factor for progression to AIDS-related death[416].

Number needed to treat

Treating individuals that fall within the high LRD score risk category with DAA treatment is further supported by the NNTT to prevent one LRD in this category. Clinical trials data have shown cure rates of >90% for the majority of new DAAs coming to market, regardless of HCV genotype, liver fibrosis staging or HIV-coinfection[446;465;658]. Therefore, in instances where HCV is the main driver of liver fibrosis progression, treatment with DAA therapy is likely to have a major effect on the rate of LRD by removing the underlying cause. Assuming that DAA therapy results in an overall 80% reduction in the rate of LRD the

NNTT to prevent one LRD in a 5-year period in the high LRD score risk category is four. This means that for every four high risk individuals treated with DAA therapy one LRD would be prevented, or postponed during the next 5-year period.

The NNTT is also low for those in the medium-high risk category where ten patents would need to be treated to prevent one LRD. However, by considering that treatment with DAAs will likely be restricted by cost and supply issues, it is easy to see why the two high risk groups would be selected for treatment above the low risk groups. Assuming an 80% reduction in LRD as a result of DAA therapy, 58 and 26 coinfected individuals in the low and medium-low risk categories would have to be treated before one LRD was prevented, respectively.

Taking a more pessimistic view of the overall effect of DAAs, it is likely that many HIV/HCV-coinfected individuals will have many factors contributing to progression of liver disease, such as on-going HBV infection or alcohol abuse as well as HCV. In this case successful treatment with DAAs will remove the contribution from HCV but the other elements may remain. Therefore, DAA treatments may reduce the rate of LRD but not by as much as anticipated given treatment success rates seen in clinical trials[446;465;658]. Assuming that DAA therapy results in an overall 50% reduction in the rate of LRD, the NNTT to prevent one LRD in the high LRD score risk category is still as low as eight. However, the NNTT rises to 102 and 45 for those in the low and medium-low risk categories, respectively.

These findings are in agreement with simulation studies of HCV-monoinfection in the resource-limited setting which show that, where the number of treatments per year is restricted, life years added by HCV treatment are maximised by treating those with F3 and F4 fibrosis only[709;710]. The distinction here in HIV/HCV-coinfection is that fibrosis staging is not the only factor required when identifying who would see the most benefit from treatment.

8.5.1 Limitations

The main limitation of this study is that as data on alcohol abuse were only added to the EuroSIDA data collection form in 2010, alcohol abuse was not included as a variable in the LRD score derivation model. Alcohol abuse is known to be a key contributor to the progression of liver disease and is known to act synergistically with HCV[673]. However, data on alcohol abuse is notoriously difficult to gather from patients in the clinical setting which is why the alcohol abuse data recently added to EuroSIDA is of a qualitative nature (see Chapter 7 Section 7.5.1)[672;674]. The number of units of alcohol consumed per week is not collected in EuroSIDA, just whether an individual is considered to be an alcohol abuser or

not. Further, the follow-up form is not filled in by the individual themselves but the clinical practitioner, which may incorporate some level of observer level variation.

However, an earlier version of the MELD score contained a component linked to alcohol consumption. The cause of cirrhosis, alcoholic or cholestatic, was originally included as a quantitative term in the MELD score but was dropped after studies determined that its exclusion had only a minimal impact on the accuracy of the model[693;701]. Therefore, although not a directly comparable score designed for use in HIV/HCV-coinfected individual specifically, the MELD score provides some evidence to suggest that adding a component on alcohol consumption to the LRD score may not greatly influence the results.

As in the previous chapter, a limitation of this study was that due to limited power it was not possible to model the individual stages of liver fibrosis, which instead were grouped as F0/F1, F2/F3 and F4 fibrosis. In addition, the fibrosis data in this study are collected from a combination of clinical procedures and biomarkers; therefore, consensus on the definitions of the stages of fibrosis is difficult to attain. However, cut-off points for low levels of fibrosis or cirrhosis, are relatively well-defined[589]. In particular, the APRI score, which is where the majority of fibrosis data are taken from in this study, has been validated in a number of studies[425;685].

The minimum duration of HCV infection was found to be an independent predictor of LRD and became a contributing component of the LRD score. The minimum duration of infection was calculated using the first available positive HCVAb or HCV RNA test result, when in reality HCV infection most likely occurred sometime prior, possibly around the time of HIV infection. However, this reflects the nature of data collection and clinical care of HIV/HCV-coinfected individuals. In most cases the true date of HCV infection will not be known. Further, the LRD score performed equally well in the SHCS validation cohort where estimated minimum HCV infection durations were considerably longer than in EuroSIDA.

Finally, data were not complete at baseline in this study. However, multiple imputations were used to impute the missing data, which is a well-known statistical technique which reduces bias when data are missing completely at random (MCAR) or missing at random (MAR)[522]. The missing data in this analysis were HCV RNA, HCV genotype, HBsAg and fibrosis staging. Section 4.4.1 of this thesis showed that missing HCV RNA data was associated with Eastern Europe. Eastern Europe is more likely to have missing data than Western Europe due to differences in the quality of clinical management. However, this does not mean that the underlying distribution of people positive or negative for HCV RNA would be different in this region. Therefore, it is reasonable to assume these data are MAR.

Similarly, any differences in the underlying distributions of HBsAg, HCV genotype and fibrosis staging between Eastern and Western Europe are likely to be explained by the observed variable region of EuroSIDA. Consequently, I believe it is reasonable to assume these data are MAR. In addition, the proportion of individuals with missing data was low for key variables such as liver fibrosis staging (21%) and HBsAg status (13%).

8.5.2 Conclusion

The costs of new DAA therapies for treatment of HCV mean that the number of treatments available will be limited in many settings, including those not traditionally seen as resource-limited. Therefore, prioritisation of those at the greatest need of therapy will be essential. The LRD score is specifically tailored to determine the risk of progression to LRD among HIV/HCV-coinfected individuals in the presence of HIV-related competing risks and demonstrated the ability to accurately differentiate between those and highest and lowest risk of progression to LRD. To my knowledge this is the first externally validated prognostic score to specifically predict LRD in HIV/HCV coinfected individuals and will serve as a useful tool in the clinical setting when deciding who to prioritise for treatment with new DAA treatments for HCV.

The performance of the LRD score in this analysis opens the door for further work on the development of an individualised LRD score which can be applied using a mobile phone application or website.

Chapter 9

Overall significance and conclusions

9.1 Summary of findings

After the introduction of combination antiretroviral therapy (cART) in the mid-1990s AIDS-related mortality started to decline dramatically in the HIV population[597]. Consequently, research focus in the HIV field has shifted towards coinfections and comorbidities. Due to shared transmission routes, coinfection between HIV and HCV is common and liver-related death (LRD) as a consequence of hepatitis coinfection has assumed increasing importance among HIV/HCV coinfected individuals[372;524;564]. The results from the analysis presented in each of my chapters is summarised below, followed by a discussion of the implications for the management of HIV/HCV coinfected individuals in Europe, along with the limitations of conducting these studies in EuroSIDA.

Chapter 4

The natural history of HCV RNA during chronic HCV infection among HIV/HCV coinfected individuals

The relationship between HCV RNA levels, LRD and other clinical outcomes has been the subject of many studies. While some studies have found no meaningful association between HCV RNA and progression to liver disease, others have reported that HCV viral load may influence the degree of liver damage[546;548]. In addition, high levels of HCV RNA have been consistently linked with a poor response to treatment for HCV using pegylated-interferon and ribavirin[436]. Therefore, factors that affect changes in HCV RNA levels are important for the clinical management of HIV/HCV coinfected individuals where interferon-based treatment remains the only therapeutic option.

This analysis described the natural history of HCV RNA in HIV/HCV coinfected individuals. The main findings of this study showed that HCV RNA levels were affected by cART. Among coinfected individuals taking cART HCV RNA levels remained stable over time; however, for coinfected individuals not taking cART HCV RNA increased by 28% per year. This finding is in agreement with other work on the topic which also found minimal increases in the region of 2% per year HCV RNA over time in HIV/HCV coinfected individuals[526]. One potential explanation how cART may be able to indirectly control HCV RNA levels is that immune reconstitution following cART may lead to an increase in

immune responses to HCV core peptides[540]. In other words, when HIV is controlled by cART the immune system is better equipped to deal with HCV RNA.

HCV genotype 1 was a significant predictor of high levels of HCV RNA at baseline in this study. Further, HCV genotype 1 was also associated with HCV RNA reaching a clinically important threshold of 800,000IU/ml and rapid increases in HCV RNA over time. An HCV viral load above 800,000IU/ml has been reported as a predictor of poor treatment response to HCV treatment with pegylated-interferon and ribavirin among HIV/HCV coinfected individuals[436]. HCV treatment success rates with pegylated-interferon and ribavirin have been shown to be consistently lower among those with HCV genotype 1 and this association between genotype 1 and higher HCV RNA levels could be partially responsible[434;435].

The findings of this analysis have potential implications for the clinical management of HIV/HCV coinfected individuals. As cART appears to be able to indirectly stabilise HCV RNA it would suggest that HIV/HCV coinfected individuals would benefit from early initiation of cART during the course of HIV infection. This strategy could prevent HCV RNA levels reaching high levels associated with a poor response to treatment with pegylated-interferon and ribavirin, which is still adopted as the standard of care in many resource-limited countries.

Chapter 5

Temporal changes and regional differences in the uptake of treatment for HCV among HIV/HCV coinfected individuals in EuroSIDA

In 2012 European AIDS Clinical Society (EACS) guidelines recommended that all HIV/HCV coinfected individuals with significant liver fibrosis should be considered for HCV treatment due to an increased risk of LRD[1]. Gold standard HCV therapy at the time consisted of pegylated-interferon and ribavirin[1]; however, treatment uptake and sustained virologic response rates were typically low among HIV/HCV coinfected individuals, mainly due to contraindications to treatment and poor adherence as a result of unpleasant side-effects[624;717].

This study described the rate of uptake of treatment for HCV with pegylated-interferon plus ribavirin among chronically infected HIV/HCV coinfected individuals in Europe between 1998 and 2010. The main finding of this analysis was that 25% of coinfected individuals in Europe were treated for HCV over the study period. Although the incidence of treatment uptake increased 27% per year between 1998 and 2007 treatment rates remained low. However, the rate of HCV treatment uptake presented here was higher than in other

studies of HCV treatment prevalence which have typically included individuals based solely on an HCVAb measurement[582;583]. One of the strengths of this analysis is that HIV/HCV coinfected individuals were selected for inclusion based on HCVAb and HCV RNA so that only chronically infected individuals, who would be considered for treatment, were included.

After the peak of 2007 there was a significant decline in the rate of HCV treatment uptake. In the late 2000s drug development for new direct-acting antivirals (DAA) for treatment of HCV was advancing rapidly. New DAA drugs were showing a vast improvement in treatment success rates compared to pegylated-interferon and ribavirin in clinical trials[445;568]. Consequently, it is possible that clinicians may have begun to defer treatment for HCV with pegylated-interferon and ribavirin in anticipation of new drugs on the horizon.

Importantly, although coinfected individuals with significant liver fibrosis were 60% more likely to be treated for HCV, there remained a significant proportion of the study population who had significant liver fibrosis but had not yet been treated for HCV. Therefore, it is important to reinforce treatment guidelines and ensure that those in need of HCV treatment are considered for therapy. However, these individuals who were indicated for therapy but not treated may have had contraindications to treatment with interferon, which highlights the need for new potent less toxic treatments for HCV.

Chapter 6

The incidence of antiretroviral drug discontinuation among HIV/HCV coinfected individuals and those with significant liver fibrosis

As a consequence of effective cART for HIV, life expectancy of HIV-positive individuals diagnosed early in the course of infection has begun to approach that of the general population[8;716]. As a result, more patients are presenting with hepatic and renal impairment requiring treatment, while some antiretroviral (ARV) drugs are known to contribute to the development of these conditions during prolonged exposure[645;718]. Therefore, pharmacokinetic considerations have become central in determining appropriate ARV dosing strategies[601]. As most ARV drugs have a metabolising contribution from the liver it is possible that liver damage as a result of HIV/HCV coinfection could result in ARV drug toxicity leading to treatment discontinuation[601].

This study described the incidence of ARV drug discontinuation due to toxicity and patient or physician choice, comparing HIV monoinfected individuals with HIV/HCV coinfected individuals. HCVAb positivity was associated with an increased risk of ARV drug discontinuation consistent with other studies of the topic[536;629;630]. Expanding the research to study the effect HCV RNA on ARV drug discontinuation showed that HIV/HCV coinfected

individuals who had cleared HCV RNA had a similar risk of ARV drug discontinuation as HIV monoinfected individuals. However, chronically infected HIV/HCV coinfected individuals had a consistently increased risk of drug discontinuation for the NRTI, NNRTI and PI ARV drug classes.

Interestingly, when additionally adjusting for liver fibrosis using the biomarker hyaluronic acid (HA), significant liver fibrosis was consistently associated with ARV drug discontinuation but the role of HCV RNA became non-significant. This would suggest that liver fibrosis, possibly caused by on-going HCV viral replication, drives the association between chronic HIV/HCV coinfection and ARV drug discontinuation and not HCV viral replication *per se*. As most ARV drugs have a metabolising contribution from the P450 enzyme system in the liver these findings suggest that liver damage as a result of HCV coinfection may inhibit ARV metabolization[601]. Inefficient drug metabolization may then lead to drug overdosing and toxicity which is clinically manifested as drug discontinuation.

These findings have implications for the management for HIV/HCV coinfected individuals. As ritonavir is known to be a powerful inhibitor of the P450 enzyme system[601], the benefits and risks of ritonavir-boosted PI-based cART regimens should be assessed in coinfected individuals, particularly those with significant liver fibrosis or cirrhosis, to avoid disruptive HIV treatment and the need to discontinue or change cART regimens.

Chapter 7

Liver-related death among HIV/HCV coinfected individuals, what are the implications for treatment with direct-acting antivirals?

Progression of liver disease is common with HCV infection and known to be accelerated in the presence of HIV coinfection[413]. However, LRD is often associated with older age as complications of HCV-related liver disease can take decades to develop[651]. Therefore, HIV/HCV coinfected individuals encounter many competing risks of death such as AIDS-related mortality, mortality associated with injecting drug use (IDU), violent death, malignancies and renal disease[373].

The recent approval of less toxic, oral, DAA drugs for HCV will see fewer HIV/HCV coinfected individuals with contraindications to treatment, while treatment success rates will be greatly improved if promising clinical trials data are repeated in the general population[446;658]. However, treatment with new DAAs will be prohibitively expensive and availability will be restricted on a cost basis in many European settings[2]. Therefore, a better understanding of the spectrum of causes of death among HIV/HCV coinfected individuals

and factors associated with LRD is essential so that expensive new DAA therapy can be prioritised for those at the greatest risk of LRD.

This study described causes of death among HIV/HCV coinfected individuals in Europe along with temporal changes in the incidence of LRD and factors associated with progression to LRD. The main findings of this analysis show that although the incidence of LRD declined between the years 2000 and 2013, LRD along with AIDS-related death remains the leading causes of mortality among HIV/HCV coinfected individuals. The decline in LRD rates over the study period appears to be partially explained by improvements in CD4 cell count and lower levels of liver fibrosis over time. This finding suggests that initiating cART early in the course of HIV infection among HIV/HCV coinfected individuals may reduce the burden of LRD by preventing time spent with low CD4 cell count, which has been strongly associated with rapid progression of liver fibrosis[414;671].

Significant liver fibrosis was found to be the strongest predictor of progression to LRD, followed by low CD4 cell count, concurrent HBV infection and duration of HCV infection. Coinfected individuals with F4 liver fibrosis had a 14% 5-year probability of LRD compared with 2% for those with F0/F1 fibrosis. Consequently, liver fibrosis levels should be considered the most important factor for determining who to prioritise for treatment with expensive DAA therapy for HCV. This finding is in agreement with another recent study of HIV/HCV coinfected individuals which found that the risk of end stage liver disease (ESLD) was minimal among those with F0/F1 fibrosis, as measured by the FIB-4 index, but that the 5-year probability of ESLD increased to 17% for those with F3/F4 fibrosis[665].

Current EACS guidelines recommend deferring treatment for HCV in those with F0/F1 liver fibrosis, whereas treatment is encouraged for those with significant liver fibrosis[416]. Given the low risk of progression to LRD seen among those with low levels of liver fibrosis in this study and the prohibitive costs of treating HCV with new DAAs[2], this study strongly supports this treatment prioritisation strategy.

Chapter 8

A validated prognostic score for estimating the risk of liver-related death among HIV/HCV coinfected individuals

As new DAA regimens for treatment HCV cost in the region of €90,000, with the expectation that all-oral regimens will be more expensive still[2], studies have begun to evaluate the cost-effectiveness of these new therapies. Studies using Markov chain models to simulate the progression of liver disease among HCV monoinfected individuals following

DAA therapy have shown the potential for large reductions in the number of individuals developing advanced liver disease[684;706]. Consequently, the reduced costs associated with treating advanced liver disease mean that most studies suggest that treatment with DAA therapy is cost-effective in the long-term[684;706].

Earlier this year the National Institute for health and Care Excellence (NICE) approved the use of sofosbuvir and simeprevir for treatment of HCV in the UK on the basis that they were cost-effective interventions[707]. However, for the first time since NICE began making cost-effectiveness recommendations the NHS postponed approval of these drugs on the basis that the upfront costs were too great to bare[708]. Given that the UK NHS considers the costs of new DAA therapy to be prohibitive, it is certain that other regions not traditionally considered to be resource-limited will also struggle to meet the costs of treatment in the short-term. Therefore, prioritisation of those at the greatest risk of LRD will be of utmost importance to ensure that the limited DAA treatments that are available are channelled to those with the most urgent need of therapy.

This study built on the analysis presented in the previous chapter, describing factors associated with progression to LRD, to construct a prognostic score for LRD to aid clinicians when making difficult decisions on whom to prioritise for treatment with new DAA therapy for HCV. The LRD prognostic score was developed in EuroSIDA and includes contributions from CD4 cell count, age, HBV coinfection, liver fibrosis levels and whether taking cART. The LRD score has also been validated on external data from the Swiss HIV Cohort Study (SHCS). The main findings of this analysis show that a simple, easy to calculate prognostic score for LRD can accurately differentiate between HIV/HCV coinfected individuals at the highest and lowest risk of LRD.

Calculation of the score allows the categorisation of coinfected individuals into low, medium-low, medium-high and high risk categories. The 5-year risk of LRD for those in the low risk category was negligible (2%); however, the 5-year risk of LRD was substantial in the medium-high and high risk categories (11% and 26%, respectively). Consequently, in an era of prioritisation for those at the greatest risk of LRD for treatment with new DAA therapies, new expensive HCV treatments should be offered to those in the medium-high and high risk categories in the first instance.

The LRD score was developed specifically to predict progression to LRD among HIV/HCV coinfected individuals in the presence of HIV-related competing risks. Consequently, it is ideally placed to help clinicians decide who to treat with new DAAs. To my knowledge this is the first prognostic score developed with the specific intention of informing who to

prioritise for treatment with DAAs. The score may prove useful to clinicians and HIV/HCV coinfected individuals who wish to assess their risk of progression to LRD.

9.2 Implications for the management of HIV/HCV coinfected individuals in Europe

The past five years have seen a revolution in treatment options available for treatment of HCV. At the onset of this thesis the gold standard treatment for HCV (both HCV-monoinfected and HIV/HCV-coinfected) consisted of pegylated-interferon and ribavirin[1]. Interferon is typically associated with unpleasant side-effects and often meant many HIV/HCV coinfected individuals would have contraindications to therapy[443]. Chapter 5 of this thesis showed that treatment rates with pegylated-interferon remain low in Europe and that there is a significant proportion of coinfected individuals with significant liver fibrosis indicating therapy yet to be treated. However, the development of potent, less toxic new DAAs have heralded a new era of treatment for HCV. The first generation DAAs telaprevir and boceprevir demonstrated vastly improved treatment success rates, with sustained virologic response (SVR) rates in the region of 60-70% for HCV genotype 1[437;438]. However, these first generation DAAs still had to be taken along with pegylated-interferon, so eligibility for treatment remained low[193].

More recently second generation DAAs have come to market which can be taken with or without pegylated-interferon[416]. These second generation DAAs, such as sofosbuvir and simeprevir, have performed remarkably well in clinical trials where treatment durations as short as 8-12 weeks have resulted in more than 90% achieving SVR seemingly regardless of HIV coinfection, HCV genotype, previous unsuccessful treatment or advanced liver fibrosis[446;658]. With yet more DAAs set to come to market in the coming months and years it now seems like appropriate highly efficacious treatment will exist for everyone who has HCV infection[446;658]. However, the costs of treatment with new DAAs are such that many European regions will not be able to afford them, including regions not traditionally considered to be resource-limited[708].

Consequently, research efforts have been shifting focus to study the cost-effectiveness of new DAAs and which patient groups should be prioritised for the treatments that are available[684;706]. The work presented here in Chapters 7 and 8 describes factors associated with progression to LRD among HIV/HCV coinfected individuals and derived a LRD prognostic score. This is the first prognostic score developed specifically to determine the risk of LRD among HIV/HCV coinfected individuals in the presence of HIV-related competing risks. Therefore, the LRD score is ideally placed to help clinicians decide who to prioritise for DAA therapy. This may prove useful in the clinical setting as individuals at the greatest need of treatment have not always been channelled for therapy.

Although DAA treatments offer the possibility of complete HCV eradication in the long-term, costs, access restrictions and reinfection mean that there is still a long way to go[719]. Therefore, the earlier work presented in this thesis has value for the management of HIV/HCV coinfected individuals, in particular where new DAA treatments are not available due to supply or cost issues. Analysis presented in this thesis has shown that treatment with cART is able to indirectly control HCV RNA among HIV/HCV coinfected individuals. Further, chronic HCV coinfection and significant liver fibrosis have been associated with an increased risk of ARV drug discontinuation. These findings reinforce the message that starting HIV treatment early during the course of infection in HIV/HCV coinfected individuals is essential. The benefits of early treatment initiation in these individuals include the indirect control of HCV RNA, prevention of time spent with low CD4 cell count and suppression of HBV DNA, where HBV coinfection is present and appropriate cART regimens are used. All of these factors should help to slow the progression of liver fibrosis while waiting for HCV treatment to become widely available[388;414].

9.3 Limitations and lessons learnt

The analysis presented in this thesis has been carried out to the best of my ability with the data that were available in EuroSIDA at the time of analysis. However, there are a number of limitations to these studies relating to the quality of data available in EuroSIDA. As described in detail in Section 3.1.1, EuroSIDA was originally set up as an HIV observational cohort study in 1994. As treatment for HIV developed over time with fewer individuals progressing to AIDS, EuroSIDA evolved to include data on comorbidities such as non-AIDS defining malignancies and coinfections such as HCV and HBV. Although EuroSIDA has been successful in shifting focus as research needs have changed over time, there are consequences in data quality that result from studying HIV/HCV coinfection in a cohort that was originally set up to study HIV alone.

Key variables in the analysis of HCV such as the level of alcohol abuse, sustained virological response (SVR) rates to HCV treatments and social factors such as active injecting drug use, quality of life and accommodation status have only recently been added to EuroSIDA or remain uncaptured. Further, other important variables in HCV research such as HCV RNA, HCV genotyping and liver fibrosis levels were not of the utmost importance during the early days of HIV research. Therefore, this information tends to be missing regularly in the early course of follow-up in EuroSIDA, with data becoming more reliable over time as HCV has assumed increasing importance in the HIV community.

In addition, EuroSIDA includes clinical centres from a wide and diverse cross-section of Europe. In particular there are a number of differences in the quality of care provided in Eastern European regions compared to Western and Northern Europe. Consequently, data quality is often worse in Eastern Europe resulting in a higher proportion of missing data from that region. These limitations and their influence on the analyses presented here are discussed in detail below.

9.3.1 Study specific limitations of EuroSIDA data in HIV/HCV coinfection

Chapter 4

Chapter 4 of this thesis analysed HCV RNA profiles and their association with HIV treatment and HCV genotype. In the introduction to this chapter, previous studies of HCV RNA are critiqued as being mainly cross-sectional in nature, while the proposed strength of my analysis is described as the long term follow-up and multiple HCV RNA measurements per person. While this analysis contained a large amount of individuals with multiple HCV

RNA measurements in comparison to previous studies, the analysis still contained many individuals with only a single HCV RNA measurement.

Out of 1,541 HIV/HCV coinfected individuals included in this study there were only 258 who had at least three HCV RNA measurements, the minimum required in order to assess non-linear trends over time. Consequently, the study lacked sufficient power to be able to describe detailed non-linear patterns in the natural history of HCV RNA. Although the linear trends described in this analysis were an important contribution to the literature, the number of individuals with only one HCV RNA measurement is a major limitation of the study.

Collection of HCV RNA data has been a priority of the data monitoring process in EuroSIDA for some time. Monitors have typically found that HCV RNA measurements are taken regularly at clinical sites, but that they are often not input to the EuroSIDA follow-up forms. This means that traditionally many HCV RNA measurements were missing from the EuroSIDA data. However, data monitoring in recent years has focused on clinical sites with a large proportion of HIV/HCV coinfected individuals, with the specific intention of identifying missing HCV RNA data. Consequently, the quality and quantity of HCV RNA data in EuroSIDA has increased over time. Unfortunately these data were not complete at the time of this analysis.

Chapter 5

This chapter looking at the uptake of treatment for HCV and the proportion of individuals who complete a full course of treatment suffers from the same issue of incomplete HCV RNA data as the previous chapter. The natural extension to this work would be to consider the rate of SVR to treatment for HCV and which factors are associated with a successful treatment outcome. However, incomplete or unreported HCV RNA measurements meant that the majority of individuals treated for HCV did not have an HCV RNA measurement available six months after completion of therapy. Therefore, it was not possible to accurately determine the rate of SVR to treatment in this population, which is a major limitation of this study.

The analysis of completion of a full course of HCV treatment was further limited by the lack of data on social factors in EuroSIDA. Completion of a course of therapy is likely to be strongly influenced by qualitative factors associated with chaotic lifestyles typically attributed to injecting drug use. Assessment of active injecting drug use has only recently been added to the EuroSIDA follow-up form and was not available at the time of analysis. Consequently, behavioural factors were estimated based on each individual's HIV transmission route, grouping people as those who acquired HIV via injecting drug use,

heterosexual contact, MSM, and haemophiliacs. For most people HIV infection will have occurred many years prior to accessing care for HCV, so this is likely to be a very poor estimation of active injecting drug use and behavioural factors affecting HCV treatment completion. Therefore, active injecting drug use is highly likely to be a confounder of this analysis and may have led to some bias in the results. In particular the rates of treatment completion in each region of EuroSIDA are likely to be affected by unknown levels of active injecting drug use.

Chapter 6

This chapter attempted to describe the association between HCV status, ARV toxicity and drug discontinuation. The findings suggest that HIV/HCV coinfected individuals with chronic infection and significant liver fibrosis are at an increased risk of ARV drug discontinuation for the PI and NRTI drug classes. However, some ARV drugs, particularly older NRTI drugs such as stavudine and didanosine, are associated with the development of liver fibrosis. The hypothesis of this analysis was that liver damage as a result of HCV infection could lead to overdosing of ARV drugs which then leads to drug discontinuation. However, it is possible that some ARVs may be causing liver fibrosis themselves which then leads to overdosing and drug discontinuation. This form of reverse causation, that essentially omits the effect HCV infection on drug discontinuation, cannot be dismissed although the design of the study should limit its likelihood.

Prospective studies accumulating follow-up over time are known to be less prone to reverse causation than retrospective studies. In this analysis liver fibrosis levels are known at the time of starting each ARV, so it is less likely that the current treatment regimen caused the liver fibrosis; however, it is possible that previous ARV regimens may have contributed to liver fibrosis. Perhaps what is more likely to be influencing the results presented here is that HIV/HCV coinfected individuals may be less tolerant to side effects associated with PI and NRTI treatments. Unfortunately, social factors such as employment and accommodation status which might help to describe this relationship, as mentioned above, are not collected in EuroSIDA and this is a limitation of the analysis.

Chapter 7

This chapter identified factors associated with progression to LRD among HIV/HCV coinfected individuals. The main aim of this analysis was to provide guidance to clinicians about the strongest predictors of LRD in order to inform their decisions about who to prioritise for treatment with new expensive treatments for HCV. Alcohol intake is known to be a key contributor to liver damage and is likely to play a major role in the progression to LRD in many cases[720]. Unfortunately, data on alcohol abuse was only added to the

EuroSIDA follow-up form in 2010 and baseline for most people in this analysis was around January 2000. Therefore, it was not possible to include alcohol intake as a covariate in this analysis which is a major limitation of this study.

The data EuroSIDA now collect on alcohol intake is qualitative in nature, simply whether the clinician filling out the follow-up form deems the individual to be an alcohol abuser or not. This method of collecting alcohol data reflects the difficulty in gleaning this information from patients. Self-reported alcohol consumption is known to be a poor estimate of true alcohol consumption due to recall bias or an unwillingness of an individual to report their true alcohol intake[672]. More accurate assessment of alcohol consumption can usually be performed using alcohol use questionnaires, such as the AUDIT-C questionnaire[721], which ask a series of questions about the frequency and quantity of alcohol consumption along with other questions which touch on the consequences of heavy drinking, such as feeling guilty after drinking, being unable to remember events from a drinking session and personal injury caused as a consequence of drinking.

This kind of information is not collected in EuroSIDA. However, if I were to start this analysis again I would strongly suggest that such a questionnaire be implemented. Accurate alcohol intake data would be of great benefit to EuroSIDA when dealing with questions of clinical outcomes in HIV/HCV coinfection, particularly because this data is typically missing from most observational cohort studies of HIV/HCV coinfection. In addition, accurate assessment of the role of alcohol intake on the rate of progression to LRD among coinfected individuals would be vital for clinicians when making tough decisions on who to treat with expensive new HCV therapies.

Chapter 8

As a natural extension to the previous chapter, this study aimed to create a prognostic risk score for progression to LRD, combining all the relevant risk factors into a single directly comparable statistic. Using the score sheet a clinician could add up the relevant score contributions to attain each individual's LRD risk score. This number can then be compared with the low, medium-low, medium-high and high risk group categories to see where the individual's risk lies and the corresponding 5-year probability of LRD. In this regard the score is designed to be a tool for use by physicians in the clinic to categorise the risk profile of their HIV/HCV coinfected individuals.

However, for the score to be used correctly clinicians would require data on age, CD4 cell count, HBV status, duration of HCV infection, HIV treatment status, and liver fibrosis staging. Fortunately, most of these factors will be collected in routine clinical care. The only

elements that may be difficult to obtain are liver fibrosis staging and duration of HCV infection. As discussed in Section 2.2.5.3, the gold standard for liver fibrosis staging is liver biopsy; however, many new techniques and biomarkers are available to estimate liver fibrosis levels. Consequently, even clinicians without with the possibility of performing a liver biopsy or Fibroscan® elastography evaluation, would be able to request standard blood work including AST and platelet count assessment in order to estimate fibrosis with the APRI score[425].

In this study duration of HCV infection was estimated using the first evidence of HCV infection, the first positive HCV antibody or HCV RNA test result, for example. Unfortunately, the date of HCV infection is not captured in EuroSIDA and using the first available test result is likely to considerably underestimate the duration of HCV infection, which is a major limitation of this analysis. However, the date of HCV infection is often unknown for many individuals and not well documented in observational research. In EuroSIDA and other European cohorts the majority of HIV/HCV coinfected individuals are injecting drug users, who are likely to have acquired HCV some time prior to study entry. Therefore, the approach taken in this analysis, using known duration of HCV infection, was derived so that duration of infection could be estimated easily in the same manor across different cohort studies.

9.3.2 Missing data

As mentioned above, a consequence of studying HIV/HCV coinfection in EuroSIDA, which was originally set up to study HIV alone, is that historical HCV data are often missing. In particular, although HCV-related data quality and quantity has improved over time in EuroSIDA, many of the studies presented here suffered from missing data on HCV RNA and liver fibrosis staging during the early stages of follow-up. Various statistical approaches have been used to accommodate these missing data, however, no statistical approach can fully compensate for an incomplete dataset.

In the first two studies of this thesis dummy missing categories have been used to account for missing data, whereby those with missing data are given a separate category in the analysis. Although this is traditionally a fairly common and simple approach for handling missing data, studies have shown that using dummy missing categories can lead to bias in parameter estimates when using statistical models[522]. At the time of analysis I decided to use this method to reduce the complexity of the analysis in what were largely descriptive studies. The majority of missing data on HCV RNA and liver fibrosis staging tends to be from the Eastern region of EuroSIDA. Therefore, the results presented in these studies would tend to be less applicable to Eastern Europe. In hindsight I believe it would have

been prudent to use multiple imputations to impute the missing data in order to reduce potential bias and improve the overall generalisability of the results.

For the final three studies multiple imputations were used to impute missing data on HCV RNA, liver fibrosis staging and HCV genotype. These data tend to be missing more often in Eastern Europe which could potentially be problematic even when using multiple imputations, which assume data are at least missing at random (MAR)[722]. However, the proportion of individuals who were positive for HCV RNA was similar in Eastern Europe and the other regions or EuroSIDA in the non-missing data, while any differences in HCV genotype and liver fibrosis levels are likely to be well explained by other covariates included in EuroSIDA such as age, HIV transmission route, liver transaminases and region of residence. Therefore, I believe it is reasonable to assume these data are MAR.

In the multiple imputations performed here three sets of imputations were used in chapter 6, while for chapters 7 and 8 there were four sets of imputation. The literature suggests that relatively few sets of imputation are required, typically three to five, in order to achieve an acceptable level of deviation between imputations and therefore an accurate representation of the uncertainty surrounding the missing data[722]. However, given the accessibility of these methods with modern computing power, it is now common for many more sets of imputation to be used. Although the relatively few sets of imputations used here are unlikely to have had a large influence on the results, in hindsight if I were to rerun these analyses I would now tend to use approximately 10 sets of imputations.

9.3.3 Observational studies

Observational studies have many benefits in clinical research. EuroSIDA in particular includes follow-up on a large number of HIV-positive individuals over a period of many years. In addition, new cohorts of individuals are added as and when required to replace those lost to follow-up or who have died. Patients are followed in routine clinical care and there are few barriers or exclusions to entry into the study. This means the study is well-placed to track the epidemiology of HIV/HCV coinfection. However, it is important to note that there are inherent limitations with all observational studies.

The limitations of each analysis presented in this thesis have been discussed in detail in each chapter and above; however, observational studies in general will always contain the possibility of bias. Although a large spectrum of variables relating to HIV and HCV are collected in EuroSIDA it is inevitable that some influences on the outcome of disease are not observed or collected. Multivariable statistical analysis can begin to account for confounding but can never account for information that is unmeasured or unknown.

In particular, social factors are likely to play a key role in many facets of HIV/HCV coinfection. This is a population that is traditionally associated with injecting drug use and these individuals are often known to have chaotic lives that will interfere with the application of appropriate treatments and clinical care. With this in mind it I cannot rule out the possibility that unmeasured confounding by factors such as residential status, employment status and active injecting drug use, which are not currently collected in EuroSIDA, will have had an effect on the results presented here.

In addition, EuroSIDA and its participating clinical centres are considered to be centres of excellence for the care and management of HIV/HCV coinfected individuals. Therefore, the inference derived from EuroSIDA data may not be generalizable to the entire population of HIV/HCV coinfected individuals in Europe.

9.3.4 EuroSIDA cohort X

The EuroSIDA study is currently recruiting a new cohort of HIV/HCV coinfected individuals. The aim is to recruit 4,500 coinfected individuals to supplement those already under follow-up in EuroSIDA and at the last count 2,500 new coinfected individuals had been recruited. In order to learn the lesson of the work presented in this thesis, a new data collection form has been implemented for cohort X which includes many variables not previously collected in EuroSIDA (hepatitis-related sections of the follow-up form are included in Appendix VII). For example, the date and mode of HCV transmission will now be collected on the data collection form. Previously only the mode of HIV transmission has been collected in EuroSIDA and this has been used to make assumptions about the mode of HCV transmission. In addition, the new data collection form will include a range of data specific to new DAA therapies include dosing schedules and HCV RNA measurements to determine SVR, along with data on alcohol abuse, active injecting drug use, liver fibrosis measurements and hepatic decompensation events.

Cohort X ensures EuroSIDA will be well-placed to continue the HIV/HCV coinfection research agenda.

9.4 Further research

The natural next step in continuing the research presented here is to enable the LRD score to be used widely in the clinical community. For broad ease of use the probability of progression to LRD is categorised as low, medium-low, medium-high and high. However, a more personalised approach would be preferable whereby an individual could work out their specific risk of progression to LRD. This requires reference against the cumulative incidence function of LRD to determine an individual risk of LRD and may not be perceived as straight forward in the clinical setting. A more user friendly approach would be to develop a website or mobile phone application where individual characteristics can be input to return a personalised risk of progression to LRD. Work will be on-going to develop these tools in the coming months and I hope to be able to make them widely available as soon as possible.

With the continuing enrolment of Cohort X EuroSIDA is well-placed to assess the impact of DAAs on the HIV/HCV coinfected community. While clinical trials results have shown the new therapies to have excellent cure rates there is often a tendency for trials to include select groups of patients that tend to perform better than the general population[723]. In addition, clinical trials rarely include long-term follow-up and the comparatively low number of individuals included mean that rare conditions that take time to develop are unlikely to be discovered. Consequently, it will be essential to monitor the uptake of treatment with DAAs and how successful treatment is in the long term. Long-term follow-up in a large number of individuals will also allow for the description of rare side effects, reinfection rates and long-term outcomes following treatment.

While DAA therapies remain prohibitively expensive it is important to continue to reaffirm the potential benefits of treatment to exert pressure on authorities to increase access. Analysis of the potential burden of liver disease among HIV/HCV coinfected individuals in Europe would help to describe the number of preventable deaths should DAA treatment become widely available. A Markov model could be used to simulate the progression of liver disease among HIV/HCV coinfected individuals. EuroSIDA would be well-placed to develop such a model as the large amount of data available on HIV/HCV coinfected individuals would allow for accurate estimation of the model's parameters and disease pathways.

As access to DAA treatment becomes more widely available research focus will likely shift towards early identification of HIV/HCV coinfected individuals. Effective treatment cannot be administered until an individual is aware of their infection. Therefore, qualitative

research will be important to discover ways of diagnosing HIV/HCV coinfection. Research in the HIV field has attempted to find ways of identifying the undiagnosed with studies assessing whether other conditions could act as indicator diseases which would trigger an HIV test. Similar work could prove to be beneficial among HIV/HCV coinfected individuals to diagnose HCV infection. In the United States the cost-effectiveness of testing the whole generation of '*baby boomers'* for HCV infection has been evaluated[724]. A similar analysis could be performed in European coinfected individuals to establish whether it would be cost-effective to test everyone of a certain age group.

9.5 Concluding remarks

The aims of this thesis were to describe epidemiological characteristics of HIV/HCV coinfection and to provide guidance on the optimum management of coinfected individuals. Treatment for HCV has undergone a revolution in the past 5 years and the outlook of treatment for HCV is now very promising. There is potential for curative treatment for all HIV/HCV coinfected individuals but the costs of treatment mean that for the time being prioritisation of those at the greatest risk of LRD is essential. The main contribution to the field of HIV/HCV research presented in this thesis is the development of a prognostic score to aid clinicians when deciding who to prioritise for new HCV treatments.

Data presented here have also shown that while access to new treatments remains restricted by costs it is important that coinfected individuals continue to receive optimal care, starting HIV treatment early in the course of infection, to avoid the rapid progression of liver fibrosis.

EuroSIDA continues to shift focus to HIV/HCV coinfection and the inception of cohort X will ensure it is well-placed to conduct important research in the field for years to come. Future work will focus on how to ensure the LRD prognostic score is easily applicable in the clinical setting and simulation of the HIV/HCV epidemic in Europe to estimate the future burden of liver disease.

Appendix I

The EuroSIDA Study Group

The multi-centre study group, EuroSIDA (national coordinators in parenthesis).

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Ospedale di Bolzano, Divisione Malattie Infettive, Bolzano, Italy
Ospedale Cotugno, III Divisione Malattie Infettive, Napoli, Italy
Hospital Carlos III, Departamento de Enfermedades Infecciosas, Madrid, Spain
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Chair: J Rockstroh
Vice-chair: S De Wit
Study Co-leads: A Mocroft, O Kirk

EuroSIDA Representatives to EuroCoord: O Kirk, A Mocroft, J Grarup, P Reiss, A Cozzi-Lepri, R Thiebaut, J Rockstroh, D Burger, R Paredes, L Peters
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Statistical Staff: A Mocroft, A Phillips, A Cozzi-Lepri, D Grint, L Shepherd, A Schultze

Appendix II

The coding of causes of death in HIV (CoDe) form

Review Form

CoDe

Study:_____

CoDe Event ID: _____

Date of death : __ __ - __ __ __ - __ __
(dd/mmm/yy eg 01-FEB-05)

Section 1 ♦ Underlying cause of death and conditions contributing to death

(Please refer to the table on page 2)

Cause of death	Illness/Condition/ Injury (text)	CoDe (01-92)	Certainty:		
			Definitely	Likely	Possibly
Immediate (Mandatory)	ICD10 code (optional) __ __ __ . __ __		☐	☐	☐
Contributing (If applicable)			☐	☐	☐
Contributing (If applicable)			☐	☐	☐
Contributing (If applicable)			☐	☐	☐
Contributing (If applicable)			☐	☐	☐
Underlying (Mandatory)	ICD10 code (optional) __ __ __ . __ __		☐	☐	☐

Comments:

Section 2 ♦ Death related to immunodeficiency?

Was the underlying or contributing cause of death a CDC C disease or Hodgkin's Lymphoma?

☐ Yes ☐ No ☐ Unknown

*If **No,***
by applying the CoDe guidelines on the next page, do you consider the death to be related to immunodeficiency?
(Please refer to the algorithm on page 2)

☐ Yes, definitively
☐ Yes, likely
☐ Yes, possibly
☐ No, assumed not
☐ No, definitely not

Comments_____

Completed by : Name (Signature) :_____

Date (dd/mmm/yy eg 01-FEB-05): __ __ - __ __ __ - __ __ **Initials:** _____

For internal use only: Reviewer ID_____Date_____

Reviewer Reference Page

CoDe

Section 1 Instructions:

The CoDe system: All of the causes should be coded by using the CoDe codes provided in the below listing. Note that the system is separated in three sections, where the first section including more specific causes takes priority over the second, which again takes priority over the third section:

Code		Description	Code		Description
01		AIDS (ongoing active disease)	**05**		Diabetes Mellitus (complication to)
	01.1	Infection	**06**		Pancreatitis
	01.2	Malignancy	**07**		Lactic acidosis
02		Infection (other than 01.1)	**08**		MI or other ischemic heart disease
	02.1	Bacterial		08.1	AMI
	02.1.1	Bacterial with sepsis		08.1.1	Definitive AMI (Dundee 1)
	02.2	Others		08.1.2	Possible AMI (Dundee 2/9)
	02.2.1	Other with sepsis		08.2	Other ischemic heart disease
	02.3	Unknown aetiology	**09**		Stroke
	02.3.1	Unknown with sepsis	**10**		Gastro-intestinal haemorrhage (if chosen, specify underlying cause)
03		Chronic viral hepatitis (progression of / complication to)	**11**		Primary pulmonary hypertension
	03.1	HCV	**12**		Lung embolus
	03.1.1	HCV with cirrhosis	**13**		Chronic obstructive lung disease
	03.1.2	HCV with liver failure	**14**		Liver failure (other than 03, 03.1, 03.2)
	03.2	HBV	**15**		Renal failure
	03.2.1	HBV with cirrhosis	**16**		Accident or other violent death (not suicide)
	03.2.2	HBV with liver failure	**17**		Suicide
04		Malignancy (other than 01.2 and 03, 03.1, 03.2)	**18**		Euthanasia
	04.03	ANUS - Anal cancer	**19**		Substance abuse (active)
	04.04	BLAD - Bladder cancer		19.1	Chronic Alcohol abuse
	04.05	BONE - Bone cancer		19.2	Chronic intravenous drug-use
	04.06	BRAC - Brain cancer		19.3	Acute intoxication (indicate agent)
	04.07	BRCA - Breast cancer	**If the cause of death can't be specifically classified, general classification can be used:**		
	04.10.1	ALL - Leukaemia: Acute lymphoid	**20**		Haematological disease (other causes)
	04.10.2	AML - Leukaemia: Acute myeloid	**21**		Endocrine disease (other causes)
	04.10.3	CLL - Leukaemia: Chronic lymphoid	**22**		Psychiatric disease (other causes)
	04.10.4	CML - Leukaemia: Chronic myeloid		22.1	Mental and behavioural disorders due to use of psychoactive substances (other than alcohol and intravenous opioids)
	04.10.9	LEUK - Leukaemia: unspecified		22.2	Schizophrenia, schizotypal and delusional Disorders
	04.11	COTC - Connective tissue cancer		22.3	Mood /Affective disorders (Major depressive disorder, Bipolar disorder and other mood disorders)
	04.12	ESOP - Esophagus cancer		22.4	Neurotic, stress-related and somatoform disorders (including anxiety disorders, phobias, OCD, stress reaction, dissociative disorders, somatoform disorders)
	04.13	GALL - Biliary tract cancer		22.5	Behavioral syndromes associated with physiological disturbances and physical factors (including eating disorders, sleep disorders, sexual disorders)
	04.14	GYCA - Gynaecologic cancer		22.90	Other psychiatric disorders
	04.15	HDL - Hodgkin lymphoma	**23**		CNS disease (other causes)
	04.16	HENE - Head and neck (incl. face) cancers		23.1	Movement disorders (Parkinson's disease; dystonias and Parkinson-like syndromes)

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CoDe

	04.17	KIDN - Kidney cancer		23.2	Degenerative disorders of the central nervous system (Alzheimer's disease; Creutzfeldt–Jakob disease and other degenerative diseases of nervous system)
	04.18	COLO - Colon cancer		23.3	Demyelinating diseases of the central nervous system (Multiple sclerosis, other demyelinating diseases)
	04.19	LIPC - Lip cancer		23.4	Epilepsy (including localised and generalized epilepsy and epileptic syndromes)
	04.20	LIVR - Liver cancer		23.5	Polyneuropathies (Guillain–Barré syndrome and other polyneuropathies/disorders of the peripheral nervous system)
	04.21	LUNG - Lung cancer		23.6	Diseases of myoneural junction and muscle (Miastenia gravis and other myoneural disorders)
	04.22	MALM - Malignant melanoma		23.90	Other disorders of the nervous system
	04.27	MULM - Multiple myeloma	**24**		Heart or vascular (other causes)
	04.29	PANC - Pancreas cancer	**25**		Respiratory disease (other causes)
	04.31	PENC - Penile cancer	**26**		Digestive system disease (other causes)
	04.32	PROS - Prostate cancer	**27**		Skin and motor system disease (other causes)
	04.33	RECT - Rectum cancer	**28**		Urogenital disease (other causes)
	04.34	STOM - Stomach cancer	**29**		Obstetric complications
	04.35	TESE - Testicular cancer	**30**		Congenital disorders
	04.36	UTER - Uterus cancer	**31**		Symptoms caused by mitochondrial toxicity (other than 06, 07)
	04.40.1	MEAC - Metastasis: of adenocarcinoma	**32**		Bleeding (haemophilia)
	04.40.2	MEOC - Metastasis: of other cancer type	**33**		Sudden Infant death
				33.1	Child Abuse
	04.40.3	MESC - Metastasis: of squamuos cell carcinoma	**If the cause of death is unclassifiable, use:**		
	04.40.9	META - Metastasis: unspecified	**90**		Other causes (provide details in Section 1)
	04.90	OTH - Other Malignancy Type	**91**		Unclassifiable causes
	04.99	UNKP - Unknown Malignancy Type	**92**		Unknown
				92.1	Unknown, competing risks

- ***Immediate cause of death:*** The disease(s) or injury directly leading to death.
- ***Contributing cause of death:*** The disease(s) or injury, which contributed to a fatal outcome.
- ***Underlying cause of death:*** The disease or injury, which initiated the train of morbid events leading directly or indirectly to death, or the circumstance of the accident or violence, which produced the fatal injury.

Please note:

Hodgkin's Lymphoma is now classified as a Non-AIDS defining malignancy.

Death reason 8 (MI or other ischemic heart disease) has been subdivided based on use of the WHO Monica Dundee score, and a sudden cardiac death category has been included and an external cardiologist will be supervising the coding of such events.

Reviewer Reference Page

CoDe

Section 2 Instructions:

*Please evaluate the relatedness of the death with immunodeficiency by using the below algorithm. The CD4 counts that should be taken into consideration are the CD4 count prior to last stopping ART, and the most recent prior to death. The former (CD4 count at last stopping ART) should be weighed the highest.

CD4 counts prior to death	***CD4 < 50 cells/µL***	***CD4 50-199 cells/µL***	***CD4 ≥ 200 cells/µL***
Sudden	**Possibly** immunodeficiency-related	**Assumed not** immunodeficiency-related	**Assumed not** immunodeficiency-related
Not sudden	**Likely** immunodeficiency-related	**Possibly** immunodeficiency-related	**Assumed not** immunodeficiency-related

'Yes, definitely': underlying or contributing cause of death a CDC C disease or Hodgkin's lymphoma

'Yes, likely', 'Yes, possibly' or 'Assumed not': see table above

*'**No, definitely not':*** the underlying, contributing and immediate causes of death are of such a nature that it is inconceivable that the person died of causes related to immunodeficiency.

Appendix III

Stability of hepatitis C virus (HCV) RNA levels among interferon-naïve HIV/HCV-coinfected individuals treated with combination antiretroviral therapy

Appendix VII

Hepatitis-related data collected in EuroSIDA Cohort X

Section B1 - Basic Clinical Information

Data entry instuctions

Test/measurement not performed:
Leave the field(s) blank.

Unknown dates:
If only the day is unknown, use the 15th and then the known month and year. For example 2007-09-15.
If both day and month are unknown, use the 1st July and then the known year. For example 2011-07-01.
If both day, month and year are unknown, use the 11th November 1911, i.e. 1911-11-11.

First seen at the department: _____

Last clinical follow-up: _____

Time of AIDS diagnosis if applicable: _____
(Please complete F1 and/or G1 if applicable.)

Weight and height

Do you have information about height and weight?
☐ No
☐ Yes

Height (in centimeters): _____
((999cm = unknown))

Enrolment weight (in kilograms): _____
((999,0=unknown))

Measurement date: _____

Blood pressure

Has the bloodpressure been measured?
☐ No
☐ Yes

Date of measurement: _____

Unit:
☐ mmHg
☐ cmHg
☐ Kpa
☐ Other

If other, please specify: _____

Systolic value: _____

Diastolic value: _____

Smoking status

Is the patient currently a cigarette smoker?
- ☐ No
- ☐ Yes
- ☐ Unknown

If NO - has he/she ever smoked cigarettes?
- ☐ No
- ☐ Yes
- ☐ Unknown

Myocardial infarction or stroke

Have any first degree relatives (genetic mother, father, brother, sister) experienced myocardial infarction or stroke before the age of 50 years?
- ☐ No
- ☐ Yes
- ☐ Unknown

Alcohol abuse

Definition:
For men: An intake of > 25 alcohol-containing units a week.
For women: An intake of > 20 alcohol-containing units a week.

Past alcohol abuse
- ☐ No
- ☐ Yes
- ☐ Unknown

Current alcohol abuse
- ☐ No
- ☐ Yes
- ☐ Unknown

Drug user

Is this patient currently an active injecting drug user (at least once within the last month)?
- ☐ No
- ☐ Yes
- ☐ Unknown

Is this patient currently receiving opiate maintenance therapy?
- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, date of starting therapy: _____

If yes, please specify type of therapy:
- ☐ Methadon
- ☐ Buprenorphine
- ☐ Buprenorphine + naloxone
- ☐ Other (slow-release morphine, injectable heroin etc.)

If other, please specify: _____

Treatment of psychiatric disorders

Is this patient currently receiving antipsychotic therapy? Antipsychotic therapy: medications to reduce the psychotic symptoms of schizophrenia and other mental illnesses.
☐ No
☐ Yes
☐ Unknown

If yes, date of starting therapy: _____

Is the patient currently receiving therapy with antidepressant drugs? Antidepressant therapy: monoamine oxidase inhibitors, tricyclic antidepressants, tetracyclic antidepressants, selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors.
☐ No
☐ Yes
☐ Unknown

If yes, date of starting therapy: _____

Is this patient currently receiving benzodiazepine therapy (daily use)?
☐ No
☐ Yes
☐ Unknown

If yes, date of starting therapy: _____

Vitamin D

Does this patient take any vitamin D supplementation?
☐ No
☐ Yes
☐ Unknown

Any use of bisphosphonates?
☐ No
☐ Yes
☐ Unknown

Please indicate the most recent value of 25 hydroxy-vitamin D:

Date of measurement: _____

Unit:
☐ nmol/l
☐ ng/ml
☐ Other

If other, please specify: _____

Value: _____

Section C - Hepatitis Virology And Serology

Data entry instructions

Test/measurement not performed:
Leave the field(s) blank.

Unknown dates:
If only the day is unknown, use the 15th and then the known month and year. For example 2007-09-15.
If both day and month are unknown, use the 1st July and then the known year. For example 2011-07-01.
If both day, month and year are unknown, use the 11th November 1911, i.e. 1911-11-11.

Anti-HCV and HBsAg tests

Has the patient had a positive anti-HCV test?
☐ No
☐ Yes
☐ Unknown

Date of first positive anti-HCV: _____

Has the patient had a positive HBsAg test?
☐ No
☐ Yes
☐ Unknown

Date of first positive HBsAg: _____

Most recent tests

Hepatitis B surface antigen (HBsAg) - DATE: _____

Hepatitis B surface antigen (HBsAg) - RESULT:
☐ Positive
☐ Negative
☐ Unknown

Hepatitis B surface antibody (HBsAb) - DATE: _____

Hepatitis B surface antibody (HBsAb) - RESULT:
☐ Positive
☐ Negative
☐ Unknown

Hepatitis B IgG core antibody (HBcIgG) - DATE: _____

Hepatitis B IgG core antibody (HBcIgG) - RESULT:
☐ Positive
☐ Negative
☐ Unknown

Hepatitis B core antibody total (IgM + IgG anti-HBc) - DATE: _____

Hepatitis B core antibody total (IgM + IgG anti-HBc) - RESULT:
☐ Positive
☐ Negative
☐ Unknown

Hepatitis C antibody (Anti-HCV IgG) - DATE: _____

Hepatitis C antibody (Anti-HCV IgG) - RESULT:
☐ Positive
☐ Negative
☐ Unknown

HCV-genotype - DATE (most recent): _____

HCV-genotype - VALUE (most recent): _____

HCV subtype (most recent): _____

Has IL28b genotype ever been tested for?
- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, what was the result?
- ☐ CC
- ☐ CT
- ☐ TT

HBV-DNA values

First measurement:

Date: _____

Result:
- ☐ Positive
- ☐ Negative
- ☐ Unknown

Value: _____

Unit:
- ☐ Copies/mL
- ☐ IU/mL
- ☐ Other

If other, please specify: _____

Below level of detection?
- ☐ No
- ☐ Yes

Detection limit: _____

Assay:
- ☐ Abbott Real Time HBV
- ☐ Roche COBAS Amplicor HBV Monitor
- ☐ Roche COBAS AmpliPrep/TaqMan HBV Test
- ☐ Siemens VERSANT HBV DNA (bDNA)
- ☐ Qiagen artus HBV PCR kit
- ☐ Other

If other, please specify - including detection limit: _____

Most recent measurement:

Date: _____

Result:
- ☐ Positive
- ☐ Negative
- ☐ Unknown

Value: _____

Unit:
- ☐ Copies/mL
- ☐ IU/mL
- ☐ Other

If other, please specify: _____

Below level of detection?
- ☐ No
- ☐ Yes

Detection limit: _____

Assay:
- ☐ Abbott Real Time HBV
- ☐ Roche COBAS Amplicor HBV Monitor
- ☐ Roche COBAS AmpliPrep/TaqMan HBV Test
- ☐ Siemens VERSANT HBV DNA (bDNA)
- ☐ Qiagen artus HBV PCR kit
- ☐ Other

If other, please specify - including detection limit: _____

HCV-RNA values

First measurement:

Date: _____

Result:
- ☐ Positive
- ☐ Negative
- ☐ Unknown

Unit:
- ☐ Copies/mL
- ☐ IU/mL
- ☐ Other

If other, please specify: _____

Value: _____

Below level of detection?
- ☐ No
- ☐ Yes

Detection limit: _____

Assay:
- ☐ Abbott Real Time HCV
- ☐ Roche COBAS Amplicor HCV Monitor
- ☐ Roche COBAS AmpliPrep/TaqMan HCV Test
- ☐ Siemens VERSANT HCV DNA (bDNA)
- ☐ Qiagen artus HCV PCR kit
- ☐ Other

If other, please specify - including detection limit: _____

Most recent measurement:

Date: _____

Result:
- ☐ Positive
- ☐ Negative
- ☐ Unknown

Unit:
- ☐ Copies/mL
- ☐ IU/mL
- ☐ Other

If other, please specify: _____

Value: _____

Below level of detection?
- ☐ No
- ☐ Yes

Detection limit: _____

Assay:

- ☐ Abbott Real Time HCV
- ☐ Roche COBAS Amplicor HCV Monitor
- ☐ Roche COBAS AmpliPrep/TaqMan HCV Test
- ☐ Siemens VERSANT HCV DNA (bDNA)
- ☐ Qiagen artus HCV PCR kit
- ☐ Other

If other, please specify - including detection limit: _____

Mode of HCV infection

Mode of HCV infection:

- ☐ Homo/bisexual man
- ☐ Injecting drug user
- ☐ Homo/bisexual man + injecting drug user
- ☐ Haemophiliac
- ☐ Transfusion recipient
- ☐ Heterosexual contact
- ☐ Other
- ☐ Unknown

If other, please specify: _____

Section F3 - Clinical Events

Data entry instructions

Test/measurement not performed:
Leave the field(s) blank.

Unknown dates:
If only the day is unknown, use the 15th and then the known month and year. For example 2007-09-15.
If both day and month are unknown, use the 1st July and then the known year. For example 2011-07-01.
If both day, month and year are unknown, use the 11th November 1911, i.e. 1911-11-11.

Clinical events

Have any of the following serious events occured? - Cardiovascular events (Carotic endarterectomy, coronary angioplasty/stenting, myocardial infarction, stroke) - Metabolic events (Diabetes Mellitus, lipodystrophy) - Other organ events (Avascular necrosis, Bone fracture, Pancreatitis, End Stage Renal Disease, Liver transplantation)

- ☐ No
- ☐ Yes
- ☐ Unknown

See definitions for section F3 under "Project Bookmarks" on the left hand side.

Note: Clicking the link will open a new window, allowing you to contiue entering data.

If yes, which?

- ☐ Cardiovascular events (Carotic endarterectomy, coronary angioplasty/stenting, myocardial infarction, stroke)
- ☐ Metabolic events (Diabetes Mellitus, lipodystrophy)
- ☐ Other organ events (Avascular necrosis, Bone fracture, Pancreatitis, End Stage Renal Disease, Liver transplantation)

Cardiovascular events

Carotic endarterectomy

- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, date of event: _____

Coronary angioplasty/stenting

- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, date of event: _____

Coronary artery-by-pass grafting

- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, date of event: _____

Myocardial infarction

- ☐ No
- ☐ Yes
- ☐ Unknown

If yes, date of event: _____

Stroke
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

Metabolic events

Diabetes Mellitus
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

Lipodystrophy

Is the patient experiencing loss of fat from extremeties, buttocks or face?
☐ No
☐ Yes
☐ Unknown

If yes, date of first onset only: _____

Is the patient experiencing accumulation of fat in abdomen, neck, breast or other defined location?
☐ No
☐ Yes
☐ Unknown

If yes, date of first onset only: _____

Other organ events

Avascular necrosis in the femural head (by imaging). Definition: Diagnosed by the combination of clinical symptoms (pain, walking difficulties) and imaging findings (MRI, bone scintigraphy)
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

Bone fracture Diagnosed by X-ray
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

If yes, please specify location: _____

Bone fracture Diagnosed by X-ray
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

If yes, please specify location: _____

Pancreatitis (symptoms + objective evidence) Definition: Typical clinical history (i.e. severe abdominal pain), plus one or more of the following: elevated serum amylase > 1.5x ULN, elevated serum lipase, radiological findings.
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

End Stage Renal Disease (dialysis/transplantation) Definition: Hemodialysis or peritoneal dialysis expected to last at least three month, documented in a clinical note or a kidney transplant, documented in a clinical note.
☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

Liver transplantation

☐ No
☐ Yes
☐ Unknown

If yes, date of event: _____

Liver related tests

Has a liver biopsy ever been performed?

☐ No
☐ Yes
☐ Unknown

Date of biopsy (1): _____

METAVIR stage (F0-F4):

☐ F0
☐ F1
☐ F2
☐ F3
☐ F4

Please enclose a copy of biopsy report (1):

Date of biopsy (2): _____

METAVIR stage (F0-F4):

☐ F0
☐ F1
☐ F2
☐ F3
☐ F4

Please enclose a copy of biopsy report (2):

Date of biopsy (3): _____

METAVIR stage (F0-F4):

☐ F0
☐ F1
☐ F2
☐ F3
☐ F4

Please enclose a copy of biopsy report (3):

Has FibroScan of the liver ever been performed?

☐ No
☐ Yes
☐ Unknown

Date of the most recent FibroScan performed: _____

Result (kPa): _____

Section F4 - Hepatic Decompensation

Data entry instructions

Test/measurement not performed:
Leave the field(s) blank.

Unknown dates:
If only the day is unknown, use the 15th and then the known month and year. For example 2007-09-15.
If both day and month are unknown, use the 1st July and then the known year. For example 2011-07-01.
If both day, month and year are unknown, use the 11th November 1911, i.e. 1911-11-11.

Hepatic decompensation

Any new or previous signs of hepatic decompensation?
- ☐ No
- ☐ Yes
- ☐ Unknown

See definitions for section F4 under "Project Bookmarks" on the left hand side.

Note: Clicking the link will open a new window, allowing you to contiue entering data.

If yes, please complete the following section:

If no, please go to section G1.

Signs of hepatic decompensation (1):
- ☐ ASCI: Ascites
- ☐ HEPA: Hepatic encephalopathy grade 3 or 4
- ☐ HESY: Hepatorenal syndrome
- ☐ OESO: Oesophageal variceal bleeding
- ☐ PERI: Spontaneous bacterial peritonitis
- ☐ Other

If other, please specify (1): _____

Date of onset (1): _____

Way of diagnosis (1):
- ☐ Definitive
- ☐ Presumptive
- ☐ Autopsy

Signs of hepatic decompensation (2):
- ☐ ASCI: Ascites
- ☐ HEPA: Hepatic encephalopathy grade 3 or 4
- ☐ HESY: Hepatorenal syndrome
- ☐ OESO: Oesophageal variceal bleeding
- ☐ PERI: Spontaneous bacterial peritonitis
- ☐ Other

If other, please specify (2): _____

Date of onset (2): _____

Way of diagnosis (2):
- ☐ Definitive
- ☐ Presumptive
- ☐ Autopsy

Sign of hepatic decompensation (3):

- ☐ ASCI: Ascites
- ☐ HEPA: Hepatic encephalopathy grade 3 or 4
- ☐ HESY: Hepatorenal syndrome
- ☐ OESO: Oesophageal variceal bleeding
- ☐ PERI: Spontaneous bacterial peritonitis
- ☐ Other

If other, please specify (3): _____

Date of onset (3): _____

Way of diagnosis (3):

- ☐ Definitive
- ☐ Presumptive
- ☐ Autopsy

Signs of hepatic decompensation (4):

- ☐ ASCI: Ascites
- ☐ HEPA: Hepatic encephalopathy grade 3 or 4
- ☐ HESY: Hepatorenal syndrome
- ☐ OESO: Oesophageal variceal bleeding
- ☐ PERI: Spontaneous bacterial peritonitis
- ☐ Other

If other, please specify (4): _____

Date of onset (4): _____

Way of diagnosis (4):

- ☐ Definitive
- ☐ Presumptive
- ☐ Autopsy

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